Thromboplastin L

REF 5265HL
REF 5265L
REF 5267L

CE IVD

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Thromboplastin L
Instructions for use — en

INTENDED PURPOSE

The Thromboplastin L kit is intended for carrying out clot based haemostasis assays.

The first standardised one-stage prothrombin time test was developed by Dr. Armand Quick in 1935. It has now become the basic coagulation screening test for the diagnosis of congential and acquired deficiencies of clotting factors from the extrinsic pathway (factors II, V, VII and X)[1,2]. It is also used for the induction and monitoring of oral anticoagulant therapy[3,4] and can be used to assess the protein synthesis capability of the liver in chronic or acute hepatic disorders.
Thromboplastin L is of rabbit brain origin but resembles human preparations in its low International Sensitivity Index (ISI). The ISI of Thromboplastin L is approximately 1.1 and is calibrated against the WHO international reference preparation[5]. Thromboplastin L is particularly suited to the monitoring of oral anticoagulant therapy and, in conjunction with the appropriate factor deficient plasma, the measurement of factor activity in the extrinsic pathway. Tissue thromboplastin, in the presence of calcium ions, is an activator which initiates the extrinsic pathway of coagulation. When a mixture of tissue thromboplastin and calcium ions is added to normal citrated plasma, the clotting mechanism is activated, leading to a fibrin clot. If a deficiency exists within the extrinsic pathway, the time required for clot formation will be prolonged depending on the severity of the deficiency.

WARNINGS AND PRECAUTIONS

The reagents contained in this kit are for *in vitro* diagnostic use only – DO NOT INGEST. Wear appropriate personal protective equipment when handling all kit components. Refer to the product safety declaration for the link to appropriate hazard and precautionary statements where applicable. Dispose of components in accordance with local regulations.

COMPOSITION

Composition	Content	Description	Preparation
Thromboplastin L	2 x 5 mL (REF 5265HL) 8 x 5 mL (REF 5265L) 10 x 10 mL (REF 5267L)	Liquid Rabbit Brain Thromboplastin containing Calcium Chloride, stabilisers and preservatives.	The liquid, calcified thromboplastin is ready-for-use. No further calcium is required to carry out standard PT Assays. The contents of the vial should be mixed well before use. (5 minutes on roller).
Each kit contains Instructions For Use.			
Each kit contains lot specific reference values insert.			

ITEMS REQUIRED BUT NOT PROVIDED

The below products can be used in conjunction with Thromboplastin L:

REF 5519	ISI Calibrant Plasma Set
REF 5490	INR Reference Set

STORAGE, SHELF-LIFE AND STABILITY

Unopened reagents are stable until the given expiry date when stored under conditions indicated on the vial or kit label.

Thromboplastin L	Opened vials are stable for 2 months at $^{+}$2 –$^{+}$8°C, 5 days at $^{+}$15°C (on-board Sysmex CA-1500) and 6 hours at $^{+}$37°C (on-board AC-4 including reagent container and cap). A shift-use stability of 7 days (Sysmex CA-1500) can be achieved. DO NOT FREEZE. Large clumps of particles or changes in expected values may indicate product deterioration.

SAMPLE COLLECTION AND PREPARATION

Plastic or siliconised glass should be used throughout. Blood (9 parts) should be collected into 3.2% or 3.8% sodium citrate anticoagulant (1 part). Separate plasma after centrifugation at 1500 x g for 15 minutes. Plasma should be kept at $^{+}$18 –$^{+}$24°C. Testing should be completed within 4 hours of sample collection, or plasma can be stored frozen at -20°C for 2 weeks or -70°C for 6 months. Thaw quickly at $^{+}$37°C prior to testing. Do not keep at $^{+}$37°C for more than 5 minutes[6].

PROCEDURE

For accurate INR reporting, it is recommended to determine the laboratory specific ISI of the reagent with the testing system in use. The Helena Biosciences Europe ISI Calibrant Plasma Set (REF 5519) is recommended for this purpose[7,8]. This should be performed for each new reagent batch. The Helena Biosciences Europe INR Reference Set (REF 5490) should be used to check for shifts in the local system ISI which have been noted with changes in laboratory temperature and post instrument servicing, amongst other local variances.

Manual Method

1. Mix sufficient Thromboplastin L to complete the anticipated testing for the day and incubate at $^{+}$37°C for no more than 4 hours.
2. Pre-warm 0.1 mL of the test plasma at $^{+}$37°C for 2 minutes.
3. Add 0.2 mL of freshly mixed thromboplastin reagent to the plasma while simultaneously starting a stopwatch.
4. Note the time for clot formation to the nearest 0.1 seconds.

Automated Method

Refer to the appropriate instrument operator manual for detailed instructions or contact Helena Biosciences Europe for instrument specific application guides.

INTERPRETATION OF RESULTS

Results should be reported to the nearest 0.1 seconds and duplicates should agree within 5% of each other. %PT values can be interpolated from the calibration graph (%PT of PT Calibration plasmas versus measured clot time), which should be a straight line when plotted on log-log graph paper.

INR values can be calculated using the following formula: INR = (PT Time Patient / Mean Normal PT Time)^{ISI}

For clear guidance on the indications for and management of patients on warfarin, please refer to The British Society for Haematology, for their most current edition of 'Guidelines on oral anticoagulation with warfarin'. At time of printing this is the 2011 fourth edition[9].

LIMITATIONS

The use of serial dilutions of a reference plasma for the %PT curve is not recommended as this can lead to discrepancies caused by the low fibrinogen in the reference plasma dilutions which are not reflected in patient samples having predominantly normal fibrinogen levels. Helena Biosciences Europe advise use of the 5504R %PT/Direct INR kit for this purpose.

QUALITY CONTROL

Each laboratory should establish a quality control program. Normal and abnormal control plasmas should be tested prior to each batch of patient samples, to ensure satisfactory instrument and operator performance. If controls do not perform as expected, patient results should be considered invalid.
Helena Biosciences Europe supplies the following controls available for use with this product:

REF 5186	Routine Control N
REF 5187	Routine Control A
REF 5183	Routine Control SA
REF 5490	INR Reference Set

REFERENCE VALUES

Reference values can vary between laboratories depending on the techniques and systems in use. For this reason each laboratory should establish its own reference ranges. This is particularly important for local ISI calibration. Using the Sysmex series of instruments, normal values ranging from 11.50 - 14.60 seconds; 0.930 - 1.160 INR; 79.10 - 112.80 %PT are typical.

PERFORMANCE CHARACTERISTICS

The following performance characteristics have been determined by Helena Biosciences Europe or their representatives using a Sysmex CA-1500 coagulation instrument. Each laboratory should establish its own performance data.

Reproducibility

Sample	Routine Control N		Routine Control A		Routine Control SA	
	SD	*CV (%)*	*SD*	*CV (%)*	*SD*	*CV (%)*
Repeatability	0.07	0.59	0.24	1.09	0.45	1.11
Between-run	0.10	0.83	0.16	0.75	0.49	1.20
Between-day	0.04	0.32	0.06	0.27	0.25	0.62
Within-device / Laboratory	0.12	1.07	0.29	1.35	0.72	1.75

Interferences

Helena Thromboplastin L is insensitive to Heparin levels of up to 2 U/mL. Using a 5% interference threshold, there is no significant interference from Haemoglobin at concentrations up to 10 g/L. Using a 5% interference threshold, there is no significant interference from Bilirubin at concentrations up to 0.5 g/L for Thromboplastin L. Lipid interference testing demonstrates that lipid levels do not directly affect the clot time of the reagent up to 3.75g/L. Lipid concentrations in excess of this prevent clot detection.

Method Comparison

Comparison of clot time in seconds and INR values were determined using Thromboplastin L and Thromboplastin LI on 268 samples. The following correlations were obtained:

Thromboplastin L (Seconds) = 0.9911x + 0.1038	r^2 = 0.9941	n = 268
Thromboplastin L (INR) = 0.9853x + 0.0261	r^2 = 0.9500	n = 268

BIBLIOGRAPHY

1. Quick AJ (1935) A Study of the Coagulation Defect in Hemophilia and Jaundice, *Am. J. Med. Sci,* **190:** 501.
2. Biggs R (1976) Human Blood Coagulation, Haemostasis and Thrombosis, 2nd Edition, Blackwell Scientific Publications, London.
3. Hirsh J, Poller L, Deykin D, Levine J, Dalen JE (1989) Optimal Therapeutic Range for Oral Anticoagulants, *Chest,* **95:** 5S-11S.
4. Poller L (1986) Laboratory Control of Anticoagulant Therapy, *Sem. Thromb. Haemostasis,* **12:** 13-19.
5. World Health Organisation (1984) Expert Committee on Biological Standards, *Technical Series,* **700:** 19.
6. Clinical and Laboratory Standards Institute (2008) Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Haemostasis Assays: Approved Guideline, 5th edn. CLSI: H21-A5.
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8. Poller L, Triplett DA, Hirsh J, Carroll J, Clarke K (1995) A comparison of lyophilised artificially depleted plasmas and lyophilised plasmas from warfarin treated patients in correcting for coagulometer effects on International Normalised Ratios, *Amer. J. Clin. Pathol*, **103:** 366-371.
9. Keeling D (2011) Guidelines on Oral Anticoagulation with warfarin: Forth Edition, *British Journal of Haematology,* **154(3):** 311-324.

Thromboplastin L
Fiche technique — fr

UTILISATION

Le kit Thromboplastin L est destiné à la réalisation des analyses de l'hémostase basées sur la formation de caillots.

La première méthode de détermination standardisée du temps de prothrombine en une étape a été développée en 1935 par le Dr. Armand Quick. Cette méthode de Quick constitue désormais l'analyse de base de la coagulation servant à diagnostiquer des anomalies des facteurs de coagulation, congénitales ou acquises, à partir de la voie extrinsèque (facteurs II, V, VII et X)[1,2]. Elle sert aussi à l'induction et au monitorage des thérapies avec anticoagulants oraux[3,4] et elle peut être utilisée pour évaluer la capacité de synthèse des protéines du foie chez les patients souffrants de troubles hépatiques chroniques ou aigus.
Le Thromboplastin L provient de cerveaux de lapin mais il ressemble au BCT humain en raison de son indice de sensibilité international (ISI) faible. L'ISI du Thromboplastin L est d'environ 1,1 et est étalonné en comparaison avec la préparation internationale de référence de l'OMS[5]. Le Thromboplastin L convient tout particulièrement au monitorage des thérapies avec anticoagulants oraux et, utilisé conjointement au plasma carencé en un facteur approprié, à la détermination de l'activité du facteur de la voie extrinsèque. La thromboplastine tissulaire, en présence d'ions calcium, est un activateur qui démarre la voie extrinsèque de la coagulation. Quand un mélange de thromboplastine tissulaire et d'ions calcium est ajouté à un plasma citraté normal, le processus de coagulation, qui doit conduire à la production d'un caillot fibreux, s'active. Si la voie extrinsèque présente une anomalie, le temps nécessaire à la formation du caillot est allongé suivant la gravité du trouble de la coagulation.

AVERTISSEMENTS ET PRÉCAUTIONS

Les réactifs du kit sont à usage diagnostique *in vitro* uniquement – NE PAS INGÉRER. Porter un équipement de protection individuelle approprié lors de la manipulation de tous les composants du kit. Consulter la fiche de données de sécurité du produit pour obtenir le lien vers les phrases de risque et les conseils de prudence le cas échéant. Éliminer les composants conformément aux églementations locales.

COMPOSITION

Composant	Contient	Description	Préparation
Thromboplastin L	2 x 5 mL (REF 5265HL) 8 x 5 mL (REF 5265L) 10 x 10 mL (REF 5267L)	Thromboplastine liquide de cerveau de lapin contenant du chlorure de calcium, des stabilisateurs et des conservateurs.	La thromboplastine liquide calcifiée est prête à l'emploi. Aucun calcium supplémentaire n'est nécessaire pour réaliser des déterminations standard du TP. Le contenu du flacon doit être bien mélangé avant utilisation (5 minutes sur un mélangeur à rouleaux).
Chaque kit contient une fiche technique.			
Chaque kit contient valeurs de référence spécifiques du lot.			

MATÉRIEL NÉCESSAIRE NON FOURNI

Les produits ci-dessous peuvent être utilisés en conjonction avec la Thromboplastin L :

REF 5519	ISI Calibrant Plasma Set
REF 5490	INR Reference Set

CONSERVATION, DURÉE DE VIE UTILE ET STABILITÉ

Les flacons de réactif non ouverts sont stables jusqu'à la date de péremption indiquée s'ils sont conservés dans les conditions indiquées sur l'étiquette du kit ou du flacon.

Thromboplastin L	Les flacons ouverts sont stables pendant 2 mois à $^{+}$2 °C – $^{+}$8 °C, pendant 5 jours à $^{+}$15 °C (à bord du Sysmex CA-1500) et pendant 6 heures à $^{+}$37 °C (à bord de l'AC-4, récipient de réactif et capuchon inclus). Il est possible d'obtenir une stabilité de période de travail de 7 jours (Sysmex CA-1500). NE PAS CONGELER. La présence de d'amas de particules ou un écart par rapport aux valeurs prévues indique une détérioration du produit.

PRÉLÈVEMENT ET PRÉPARATION DES ÉCHANTILLONS

Utiliser tout au long du prélèvement du plastique ou du verre siliconé. Mélanger 9 volumes de sang et 1 volume de citrate de sodium à 3,2% ou 3,8%. Séparer le plasma après centrifugation à 1500 x g pendant 15 minutes. Conserver le plasma entre $^{+}$18 –$^{+}$24°C. L'analyse doit être terminée dans les 4 heures suivant le prélèvement de l'échantillon ; sinon, il est possible de congeler le plasma 2 semaines à -20°C ou 6 mois à -70°C. Décongeler rapidement à $^{+}$37°C avant de réaliser l'analyse. Ne pas laisser à $^{+}$37°C plus de 5 minutes[6].

PROCÉDURE

Pour obtenir un RNI (rapport normalisé international) précis, il est recommandé à chaque laboratoire de déterminer l'ISI spécifique du réactif avec le système d'analyse utilisé. Il est conseillé d'utiliser le ISI Calibrant Plasma Set Helena Biosciences Europe (REF 5519) pour cela[7,8]. Cette opération doit être réalisée pour chaque nouveau lot de réactif. Le kit RNI de référence Helena Biosciences Europe (REF 5490) doit être utilisé pour vérifier l'existence d'un décalage de l'ISI du système local déterminé en raison d'une variation de la température du laboratoire, suite à une opération de maintenance réalisée sur l'instrument ou toute autre variable locale.

Méthode Manuelle

1. Mélanger du Thromboplastin L en quantité suffisante pour réaliser les analyses prévues dans la journée et incuber à $^{+}$37 °C pendant 4 heures au maximum.
2. Préchauffer 0,1 mL de plasma à $^{+}$37°C pendant 2 minutes.
3. Ajouter 0,2 mL de réactif de thromboplastine fraîchement mélangé au plasma et démarrer à ce moment un chronomètre.
4. Relever le temps de formation du caillot en arrondissant au dixième de seconde.

Méthodes Automatisées

Consulter le manuel d'utilisation de l'instrument approprié pour obtenir des instructions détaillées ou contacter Helena Biosciences Europe pour obtenir des notes d'application spécifiques à l'instrument.

INTERPRÉTATION DES RÉSULTATS

Les résultats doivent être indiqués en arrondissant au dixième de seconde et l'écart maximal entre eux est de 5%. Il est possible d'interpoler les valeurs de %TP à partir de la courbe d'étalonnage (%TP des plasmas d'étalonnage du TP par rapport au temps de coagulation mesuré), qui doit correspondre à une ligne droite quand elle est représentée sur du papier logarithmique.

La formule suivante permet de calculer les valeurs du RNI: RNI = (Temps TP patient / Temps TP moyen normal)^{ISI}

Pour obtenir des informations claires quant aux indications et à la prise en charge des patients sous warfarine, consulter la British Society for Haematology pour obtenir la dernière édition des Guidelines on oral anticoagulation with warfarin (Recommandations relatives à l'anticoagulothérapie orale avec la warfarine). Au moment de l'impression du présent document, il s'agit de la quatrième édition de 2011[9].

LIMITES

Il est déconseillé de réaliser des dilutions du plasma de référence pour la courbe de %TP, car cela risquerait d'entraîner des divergences dues au faible taux de fibrinogène présent dans les dilutions du plasma de référence, ce qui ne serait pas représentatif des échantillons patients qui ont principalement des taux de fibrinogène normaux. Helena Biosciences Europe conseille l'utilisation du kit 5504R %PT/Direct INR dans ce but.

CONTRÔLE QUALITÉ

Chaque laboratoire doit établir un programme de contrôle qualité. Les plasmas de contrôle, normaux et anormaux, doivent être testés avant chaque lot d'échantillons patients afin de s'assurer que l'instrument et l'opérateur offrent des performances satisfaisantes. Si les contrôles ne donnent pas les résultats prévus, les résultats du patient doivent être considérés comme non valables.
Helena Biosciences Europe distribue les contrôles suivants à utiliser avec ce produit:

REF 5186	Routine Control N
REF 5187	Routine Control A
REF 5183	Routine Control SA
REF 5490	INR Reference Set

VALEURS DE RÉFÉRENCE

Les valeurs de référence peuvent varier d'un laboratoire à l'autre suivant les techniques et les systèmes utilisés. C'est pour cette raison qu'il appartient à chaque laboratoire de déterminer ses propres plages de référence. Ceci est particulièrement important pour l'étalonnage de l'ISI local. Avec les instruments de la série Sysmex, l'intervalle type des valeurs normales est de 11,50 - 14,60 secondes; 0.930 - 1.160 INR; 79.10 - 112.80 %PT .

CARACTÉRISTIQUES DE PERFORMANCES

Helena Biosciences Europe ou ses mandataires ont déterminé les caractéristiques de performance suivantes en utilisant un instrument de coagulation Sysmex CA-1500. Chaque laboratoire doit établir ses propres données de performance.

Reproductibilité

Échantillon	Routine Control N		Routine Control A		Routine Control SA	
	SD	*CV (%)*	*SD*	*CV (%)*	*SD*	*CV (%)*
Répétabilité	0,07	0,59	0,24	1,09	0,45	1,11
Inter-séries	0,10	0,83	0,16	0,75	0,49	1,20
Inter-jours	0,04	0,32	0,06	0,27	0,25	0,62
Intra-dispositif/laboratoire	0,12	1,07	0,29	1,35	0,72	1,75

Interférences

Thromboplastin L (ISI faible) d'Helena ne présente pas d'interférences avec un taux d'héparine jusqu'à 2 U/mL. En utilisant un seuil d'interférence de 5 %, il n'y a pas d'interférences significatives de l'hémoglobine à une concentration jusqu'à 10 g/L. En utilisant un seuil d'interférence de 5 %, il n'y a pas d'interférences significatives de la bilirubine à une concentration jusqu'à 0,5 g/L pour la Thromboplastin L. Une évaluation de l'interférence des lipides montre que les taux de lipides n'affectent pas le temps de coagulation du réactif jusqu'à 3,75 g/L. Une concentration en lipides dépassant cette limite empêche la détection du caillot.

Comparaison de la méthode

Des comparaisons du temps de coagulation en secondes et en valeurs de RNI ont été déterminées en utilisant les produits Thromboplastin L et Thromboplastin LI avec 268 échantillons. Les corrélations suivantes ont été obtenues:

Thromboplastin L (seconde) = 0,9911x + 0,1038	r^2 = 0,9941	n = 268
Thromboplastin L (INR) = 0,9853x + 0,0261	r^2 = 0,9500	n = 268

BIBLIOGRAPHIE

1. Quick AJ (1935) A Study of the Coagulation Defect in Hemophilia and Jaundice, *Am. J. Med. Sci,* **190:** 501.
2. Biggs R (1976) Human Blood Coagulation, Haemostasis and Thrombosis, 2nd Edition, Blackwell Scientific Publications, London.
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6. Clinical and Laboratory Standards Institute (2008) Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Haemostasis Assays: Approved Guideline, 5th edn. CLSI: H21-A5.
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9. Keeling D (2011) Guidelines on Oral Anticoagulation with warfarin: Forth Edition, *British Journal of Haematology,* **154(3):** 311-324.

Thromboplastin L
Anleitung — de

VERWENDUNGSZWECK

Das Thromboplastin L Kit ist für koagulometrische Gerinnungstests vorgesehen.

Die erste standardisierte Prothrombinzeit als Einstufen-Test wurde 1935 von Dr. Armand Quick entwickelt. Mittlerweile ist er der Standard-Screeningtest in der Gerinnung zur Diagnose eines vererbten oder erworbenen Gerinnungsfaktormangels des extrinsischen Systems (Faktor II, V, VII und X)[1,2]. Er wird auch zur Einstellung und Überwachung von oralen Antikoagulanztherapien[3,4] verwendet und kann bei chronischen und akuten Lebererkrankungen zur Beurteilung der Funktionsfähigkeit der Leber bei der Proteinsynthese herangezogen werden.
Thromboplastin L wird aus Kaninchenhirn gewonnen, ähnelt aber mit seinem niedrigen „International Sensitivity Index" (ISI) humaner Blutgerinnungszeit. Der ISI von Thromboplastin L liegt bei ca. 1,1 und ist gegen WHO „International Reference Preparation" kalibriert[5]. Thromboplastin L ist besonders für die Überwachung oraler Antikoagulanztherapien geeignet und, in Verbindung mit dem entsprechenden Faktor-Mangelplasma, bei der Messung von Faktoraktivitäten im extrinsischen System. Gewebethromboplastin ist in Anwesenheit von Calcium-Ionen ein Aktivator, der das extrinsische Gerinnungssystem auslöst. Gibt man eine Mischung von Gewebethromboplastin und Calcium-Ionen zu normalem Citratplasma, wird die Gerinnungskaskade aktiviert und es bildet sich ein Fibringerinnsel. Besteht innerhalb des extrinsischen Systems ein Mangel, verlängert sich, je nach Schwere dieses Mangels, die Zeit bis zur Ausbildung eines Gerinnsels.

WARNHINWEISE UND VORSICHTSMASSNAHMEN

Die in diesem Kit enthaltenen Reagenzien sind ausschließlich für die Verwendung von *in-vitro*-Diagnosen vorgesehen. NICHT VERSCHLUCKEN. Tragen Sie beim Umgang mit sämtlichen Komponenten des Kits geeignete Schutzausrüstung. Beachten Sie gegebenenfalls die Verweise auf entsprechende Gefahren- und Vorbeugeerklärungen in der Produktsicherheitserklärung. Entsorgen Sie die Komponenten gemäß den örtlichen Vorschriften.

ZUSAMMENSETZUNG

Komponente	Inhalt	Beschreibung	Vorbereitung
Thromboplastin L	2 x 5 mL (REF 5265HL) 8 x 5 mL (REF 5265L) 10 x 10 mL (REF 5267L)	Flüssiges Thromboplastin aus Hasenhirn mit Calciumchlorid, Stabilisatoren und Konservierungsstoffen.	Das flüssige, kalzifizierte Thromboplastin ist einsatzbereit. Für die Durchführung von standardmäßigen PT-Analysen ist kein zusätzliches Calcium erforderlich. Der Inhalt der Ampulle muss vor der Verwendung gut gemischt werden (5 Minuten auf dem Walzenmischer).
Jedes Kit enthält eine Gebrauchsanweisung			
Jedes Kit enthält chargenspezifischen Referenzwerten			

ERFORDERLICHE, ABER NICHT MITGELIEFERTE ARTIKEL

Die folgenden Produkte können in Kombination mit Thromboplastin L verwendet werden:

REF 5519	ISI Calibrant Plasma Set
REF 5490	INR Reference Set

LAGERUNG, HALTBARKEIT UND STABILITÄT

Ungeöffnete Reagenzien sind unter den auf Verpackung oder Fläschchen angegebenen Lagerbedingungen bis zum aufgedruckten Verfallsdatum stabil.

Thromboplastin L	Offene Ampullen sind 2 Monate bei $^{+}$2 –$^{+}$8°C, 5 Tage bei $^{+}$15°C (im Sysmex CA-1500) und 6 Stunden bei $^{+}$37°C (im AC-4 mit Reagenzbehälter und Deckel) stabil. Eine Stabilität von 7 Tagen im Schichteinsatz (Sysmex CA-1500) kann erreicht werden. NICHT EINFRIEREN. Große Verklumpungen oder Veränderungen in den Normalwerten können auf eine Verfall des Produkts hinweisen.

PROBENENTNAHME UND VORBEREITUNG

Nur Plastik oder Silikonglas verwenden. Blut (9 Teile) sollte in 3,2% oder 3,8% Natriumcitrat als Antikoagulanz (1 Teil) entnommen werden. 15 Minuten bei 1500 g zentrifugieren und Plasma abpipettieren. Plasma bei $^{+}$18 –$^{+}$24°C lagern. Plasma sollte innerhalb von 4 Stunden verarbeitet oder tief gefroren bei -20°C für 2 Wochen oder -70°C für 6 Monat gelagert werden. Vor dem Testen schnell bei $^{+}$37°C auftauen. Nicht länger als 5 Minuten bei $^{+}$37°C belassen[6].

VORGEHENSWEISE

Um einen korrekten INR zu erhalten wird empfohlen, den laborspezifischen ISI für das Reagenz mit dem verwendeten Testsystem zu bestimmen. Helena Biosciences Europe empfiehlt zu diesem Zweck das Plasma-Kalibrator-Set REF 5519[7,8]. Das sollte mit jeder neuen Reagenzien-Charge durchgeführt werden. Das Helena Biosciences Europe INR Reference Set (REF 5490) dient zur Überprüfung von Verschiebungen im ISI vor Ort, die unter anderem durch Veränderungen der Labortemperatur und nach Wartungsarbeiten an den Geräten festgestellt worden sind.

Manuelle Methode

1. Mischen Sie eine ausreichende Menge von Thromboplastin L an, um die für den Tag geplanten Tests durchzuführen und inkubieren Sie es nicht länger als 4 Stunden bei $^{+}$37°C.
2. 0,1 mL des zu testenden Plasmas bei $^{+}$37°C 2 Minuten vorwärmen.
3. 0,2 mL des frisch zubereiteten Thromboplastin-Reagenz zum Plasma pipettieren, dabei gleichzeitig Stoppuhr drücken.
4. Die Zeit bis zur Gerinnselbildung bis auf 0,1 Sekunden genau stoppen.

Automatisierte Methoden

Siehe die Bedienungsanleitung des entsprechenden Geräts für genaue Anweisungen oder wenden Sie sich an Helena Biosciences Europe für spezielle anwendungstechnische Hinweise.

INTERPRETATION DER ERGEBNISSE

Ergebnisse sollten bis auf 0,1 Sekunden genau gemessen werden und Doppelbestimmungen nicht mehr als 5% abweichen. % TPZ-Werte können ausgehend von der Kalibratorkurve interpoliert werden (% TPZ des TP-Kalibrationsplasmas zu gemessener Gerinnungszeit) und sollten auf doppeltlogarithmischem Millimeterpapier eine gerade Linie ergeben.

INR-Werte können anhand der folgenden Formel berechnet werden: INR = (PT Zeit Patient / Mittelwert normale TPZ)^{ISI}

Für klare Leitlinien zu den Indikationen für und der Versorgung von Patienten unter Warfarin siehe die aktuelle Ausgabe der "Guidelines on oral anticoagulation with warfarin" der British Society for Haematology. Zum Zeitpunkt der Drucklegung ist das die 4. Ausgabe, 2011[9].

EINSCHRÄNKUNGEN

Die Verwendung einer Serienverdünnung von Referenzplasma ist für die %PT-Kurve nicht zu empfehlen, da das zu Diskrepanzen aufgrund von niedrigem Fibrinogen in den Referenzplasma-Verdünnungen führen kann, das nicht den Patientenproben entspricht, die in der Regel normale Fibrinogen-Werte haben. Helena Biosciences Europe empfiehlt zu diesem Zweck das Set 5504R %PT/Direct INR einzusetzen.

QUALITÄTSKONTROLLE

Jedes Labor muss für eine eigene Qualitätskontrolle sorgen. Vor jeder Testreihe mit Patientenproben müssen normale und pathologische Kontrollplasmen getestet werden, um eine zufrieden stellende Geräteleistung und Bedienung zu gewährleisten. Liegen die Kontrollen außerhalb des Normbereichs, sind die Patientenergebnisse nicht zu verwenden.
In Verbindung mit diesem Produkt bietet Helena Biosciences Europe die folgenden Kontrollen an:

REF 5186	Routine Control N
REF 5187	Routine Control A
REF 5183	Routine Control SA
REF 5490	INR Reference Set

REFERENZWERTE

Referenzwerte können je nach Technik und verwendetem System von Labor zu Labor unterschiedlich sein. Aus diesem Grund sollte jedes Labor seine eigenen Referenzwertbereiche erstellen. Dieses ist besonders für die ISI-Kalibration vor Ort wichtig. Mit der Instrumentenreihe Sysmex liegen die typischen Normwerte zwischen 11,50 - 14,60 Sekunden; 0.930 - 1.160 INR; 79.10 - 112.80 %PT .

LEISTUNGSMERKMALE

Die folgenden Leistungseigenschaften wurden von Helena Biosciences Europe oder in ihrem Auftrag mit einem Sysmex CA-1500 Gerinnungsgerät ermittelt. Jedes Labor muss seine eigenen Werte ermitteln.

Reproduzierbarkeit

Probe	Routine Control N		Routine Control A		Routine Control SA	
	SD	*CV (%)*	*SD*	*CV (%)*	*SD*	*CV (%)*
Wiederholbarkeit	0,07	0,59	0,24	1,09	0,45	1,11
Bei verschiedenen Analysen	0,10	0,83	0,16	0,75	0,49	1,20
An verschiedenen Tagen	0,04	0,32	0,06	0,27	0,25	0,62
Selbes Gerät/Labor	0,12	1,07	0,29	1,35	0,72	1,75

Störungen

Helena Thromboplastin L ist unempfindlich gegenüber Heparinwerten bis zu 2 Einheiten/mL. Mit einer Störschwelle von 5 % gibt es keine besondere Interferenz durch Hämoglobin bei Konzentrationen von bis zu 10 g/l. Mit einer Störschwelle von 5 % gibt es keine besondere Interferenz durch Bilirubin bei Konzentrationen von bis zu 0,5 g/l bei Thromboplastin L. Lipidinterferenztests zeigen, dass die Lipidmenge die Gerinnungszeit des Reagenz bis 3,7 g/l nicht direkt beeinflusst. Höhere Lipidkonzentrationen verhindern die Gerinnungserkennung.

Methodenvergleich

Thromboplastin L und Thromboplastin LI wurden an 268 Proben angewandt. Dabei wurden die Gerinnungszeit in Sekunden verglichen und die INR-Werte bestimmt. Es ergaben sich folgende Korrelationen:

Thromboplastin L (sekunden) = 0,9911x + 0,1038	r^2 = 0,9941	n = 268
Thromboplastin L (INR) = 0,9853x + 0,0261	r^2 = 0,9500	n = 268

LITERATURVERZEICHNIS

1. Quick AJ (1935) A Study of the Coagulation Defect in Hemophilia and Jaundice, *Am. J. Med. Sci,* **190:** 501.
2. Biggs R (1976) Human Blood Coagulation, Haemostasis and Thrombosis, 2nd Edition, Blackwell Scientific Publications, London.
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9. Keeling D (2011) Guidelines on Oral Anticoagulation with warfarin: Forth Edition, *British Journal of Haematology,* **154(3):** 311-324.

Thromboplastin L
Istruzioni per l'uso

it

SCOPO PREVISTO

III kit Thromboplastin L è concepito per l'esecuzione di dosaggi di emostasi basati sulla presenza di coaguli.

I primo test del tempo di protrombina standardizzato venne messo a punto dal Dr. Armand Quick nel 1935. Attualmente, questo test è diventato il metodo basilare di screening della coagulazione per la diagnosi di deficienze congenite ed acquisite dei fattori di coagulazione dal percorso estrinseco (fattori II, V, VII e X)[1,2]. Questo test viene utilizzato anche per l'induzione e il monitoraggio della terapia anticoagulante orale[3,4] e può essere impiegato per valutare la capacità di sintesi proteica del fegato in disordini epatici cronici o acuti.
Il kit Thromboplastin L è realizzato a partire da cervello di coniglio, ma rassomiglia a BCT umano in termini di basso indice di sensibilità internazionale (ISI). L'ISI del kit Thromboplastin L è approssimativamente pari a 1,1 ed è calibrato rispetto alla preparazione di riferimento internazionale dell'OMS[5]. Il kit Thromboplastin L è particolarmente indicato per il monitoraggio della terapia anticoagulante orale e, in combinazione con plasma carente del fattore appropriato, per la misurazione dell'attività del fattore nel percorso estrinseco. In presenza di ioni di calcio, la tromboplastina tissutale è un attivatore che dà inizio al percorso di coagulazione estrinseco. Quando una miscela di tromboplastina tissutale e di ioni di calcio viene aggiunta a normale plasma citrato, si attiva il meccanismo di coagulazione che porta alla formazione di un coagulo di fibrina. Qualora sussista una deficienza all'interno del percorso estrinseco, il tempo richiesto per la formazione del coagulo risulterà prolungato in funzione della gravità della deficienza.

AVVERTENZE E PRECAUZIONI

I reagenti contenuti in questo kit sono destinati esclusivamente alla diagnostica *in vitro* - NON INGERIRE. Indossare un'adeguata attrezzatura protettiva personale durante la manipolazione di tutti i componenti del kit. Per conoscere i relativi simboli precauzionali e di pericolo, laddove pertinente, fare riferimento alla dichiarazione di sicurezza del prodotto. Smaltire i componenti conformemente alle normative locali vigenti.

COMPOSIZIONE

Componente	Contiene	Descrizione	Preparazione
Thromboplastin L	2 x 5 mL (REF 5265HL) 8 x 5 mL (REF 5265L) 10 x 10 mL (REF 5267L)	Tromboplastina liquida di cervello di coniglio contenente cloruro di calcio, stabilizzatori e conservanti.	La tromboplastina calcica liquida è pronta all'uso. Per eseguire dosaggi PT standard non è necessario altro calcio. Il contenuto della fiala deve essere miscelato accuratamente prima dell'uso (5 minuti su un rullo).

Ogni kit contiene un Istruzioni per l'uso.

Ogni kit contiene un inserto recante i valori di riferimento specifici per il lotto.

MATERIALI NECESSARI, MA NON IN DOTAZIONE

In combinazione con la Thromboplastin L è possibile utilizzare i seguenti prodotti:

REF 5519	ISI Calibrant Plasma Set
REF 5490	INR Reference Set

CONSERVAZIONE, VITA UTILE E STABILITÀ

I reagenti non aperti sono stabili fino alla data di scadenza indicata se conservati nelle condizioni riportate sul flacone o sull'etichetta del kit

Thromboplastin L — Le fiale aperte sono stabili per 2 mesi ad una temperatura compresa tra $^{+}$2 e $^{+}$8°C, per 5 giorni a $^{+}$15°C (Sysmex CA-1500 on-board) e per 6 ore a $^{+}$37°C (AC-4 on-board compresi il contenitore del reagente e il tappo). È possibile ottenere una stabilità d'uso di 7 giorni (Sysmex CA-1500).
NON CONGELARE. Ammassi consistenti di particelle o variazioni nei valori previsti possono essere indice di deterioramento del prodotto.

RACCOLTA E PREPARAZIONE DEI CAMPIONI

Nel corso dell'intera procedura è necessario utilizzare plastica o vetro siliconizzato. Il sangue (9 parti) deve essere raccolto in sodio citrato al 3,2% o al 3,8% come anticoagulante (1 parte). Separare il plasma in seguito a centrifugazione a 1500 x g per 15 minuti. Il plasma deve essere conservato a $^{+}$18 –$^{+}$24°C. I test devono essere completati entro 4 ore dalla raccolta dei campioni; in alternativa, il plasma può essere conservato congelato a -20°C per 2 settimane o a -70°C per 6 mese. Decongelare rapidamente a $^{+}$37°C prima di eseguire i test. Non conservare a $^{+}$37°C per oltre 5 minuti[6].

PROCEDURA

Per un rilevamento accurato dell'INR si raccomanda di determinare l'ISI specifico del laboratorio per il reagente con il sistema di test in uso. A tale scopo si raccomanda il ISI Calibrant Plasma Set (REF 5519) di Helena Biosciences Europe[7,8]. Questa procedura deve essere eseguita per ogni nuovo lotto di reagente. L'INR Reference Set (REF 5490) di Helena Biosciences Europe deve invece essere utilizzato per rilevare eventuali spostamenti dell'ISI del sistema locale osservati in concomitanza con cambiamenti della temperatura del laboratorio e in seguito a manutenzione dello strumento, tra le altre variazioni locali.

Metodo Manuale

1. Miscelare un quantitativo di Thromboplastin L sufficiente a completare i test previsti per la giornata e incubare a +37°C per un massimo di 4 ore.
2. Preriscaldare 0,1 mL di plasma di prova a $^{+}$37°C per 2 minuti.
3. Aggiungere al plasma 0,2 mL di reagente a base di tromboplastina appena miscelato, azionando contemporaneamente un cronometro.
4. Annotare il tempo di formazione del coagulo con un'approssimazione a 0,1 secondi.

Metodo Automatico

Fare riferimento al manuale utente dello strumento appropriato per istruzioni dettagliate oppure contattare Helena Biosciences Europe per le note applicative specifiche dello strumento.

INTERPRETAZIONE DEI RISULTATI

I risultati devono essere indicati con un'approssimazione a 0,1 secondi e le ripetizioni devono corrispondere con una tolleranza del 5%. I valori di %PT possono essere interpolati dal grafico di calibrazione (%PT dei plasmi di calibrazione PT vs. tempo di coagulazione rilevato), che, se tracciato su carta a doppia scala logaritmica, deve apparire sotto forma di linea retta.

I valori di INR possono essere calcolati utilizzando la seguente formula: INR = (Tempo di PT Paziente / Tempo di PT normale medio)^{ISI}

Per una guida chiara sulle indicazioni per la gestione dei pazienti con warfarina fare riferimento a The British Society for Haematology per la loro edizione più aggiornata delle 'Linee guida sull'anticoagulazione orale con warfarina'. Al momento della stampa questa è la quarta edizione del 2011[9].

LIMITAZIONI

Si sconsiglia l'impiego di diluizioni seriali di un plasma di riferimento per la curva %PT, che infatti possono dare luogo a discrepanze dovute al basso livello di fibrinogeno nelle diluizioni del plasma di riferimento, che non compaiono invece nei campioni dei pazienti con livelli di fibrinogeno prevalentemente normali. Helena Biosciences Europe consiglia di utilizzare a questo scopo il kit 5504R %PT/Direct INR.

CONTROLLO QUALITÀ

Ogni laboratorio deve definire un programma di controllo qualità. I plasmi di controllo normali e anormali devono essere testati prima di ogni lotto di campioni di pazienti, per garantire un livello prestazionale soddisfacente sia per quanto riguarda lo strumento che per l'operatore. Qualora i controlli non funzionassero come previsto, i risultati relativi ai pazienti dovranno essere considerati non validi.
Helena Biosciences Europe mette a disposizione i seguenti controlli utilizzabili con questo prodotto:

REF 5186	Routine Control N
REF 5187	Routine Control A
REF 5183	Routine Control SA
REF 5490	INR Reference Set

VALORI DI RIFERIMENTO

Per la sicurezza del paziente, è necessario che il sistema sia monitorato continuamente da un operatore qualificato. Per tale motivo ciascun laboratorio dovrà elaborare i propri range di riferimento. Ciò è particolarmente importante per la calibrazione dell'ISI locale. Con l'impiego della gamma di strumenti Symex, i valori normali che variano tra 11,50 - 14,60 secondi; 0.930 - 1.160 INR; 79.10 - 112.80 %PT sono ritenuti tipici.

CARATTERISTICHE PRESTAZIONALI

Le seguenti caratteristiche prestazionali sono state determinate da Helena Biosciences Europe o dai propri rappresentanti con l'utilizzo di uno strumento di coagulazione Sysmex CA-1500. Ciascun laboratorio dovrà pertanto elaborare i propri dati prestazionali.

Riproducibilità

Campione	Routine Control N		Routine Control A		Routine Control SA	
	SD	*CV (%)*	*SD*	*CV (%)*	*SD*	*CV (%)*
Ripetibilità	0,07	0,59	0,24	1,09	0,45	1,11
Tra le serie	0,10	0,83	0,16	0,75	0,49	1,20
Tra giorni	0,04	0,32	0,06	0,27	0,25	0,62
All'interno del dispositivo/laboratorio	0,12	1,07	0,29	1,35	0,72	1,75

Interferenze

La Thromboplastin L Helena non è sensibile ai livelli di eparina di oltre 2 U/mL. Utilizzando una soglia di interferenza del 5%, non risulta esserci alcuna significativa interferenza da parte dell'emoglobina a concentrazioni fino a 10 g/l. Utilizzando una soglia di interferenza del 5%, non risulta esserci alcuna significativa interferenza da parte della bilirubina a concentrazioni fino a 0,5 g/l per la Thromboplastin L. I test per le interferenze dei lipidi dimostrano che i livelli dei lipidi non influenzano direttamente il tempo di coagulazione del reagente fino a 3,75 g/l. Concentrazioni lipidiche superiori a questo valore impediscono il rilevamento del coagulo.

Confronto dei metodi

Si è eseguito un confronto su 268 campioni tra il tempo di coagulazione in secondi e i valori INR utilizzando la tromboplastina L e la tromboplastina LI. Si sono ottenute le seguenti correlazioni:

Thromboplastin L (secondi) = 0,9911x + 0,1038	r^2 = 0,9941	n = 268
Thromboplastin L (INR) = 0,9853x + 0,0261	r^2 = 0,9500	n = 268

BIBLIOGRAFIA

1. Quick AJ (1935) A Study of the Coagulation Defect in Hemophilia and Jaundice, *Am. J. Med. Sci,* **190:** 501.
2. Biggs R (1976) Human Blood Coagulation, Haemostasis and Thrombosis, 2nd Edition, Blackwell Scientific Publications, London.
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5. World Health Organisation (1984) Expert Committee on Biological Standards, *Technical Series,* **700:** 19.
6. Clinical and Laboratory Standards Institute (2008) Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Haemostasis Assays: Approved Guideline, 5th edn. CLSI: H21-A5.
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9. Keeling D (2011) Guidelines on Oral Anticoagulation with warfarin: Forth Edition, *British Journal of Haematology,* **154(3):** 311-324.

Thromboplastin L
Instrucciones de uso

es

USO PREVISTO

El uso previsto del kit Thromboplastin L es realizar ensayos de hemostasia basados en la coagulación.

La primera prueba estandarizada de la protrombina en una sola etapa fue desarrollada por el Dr. Armand Quick en 1935. Ahora se ha convertido en la prueba de cribado básico de la coagulación para el diagnóstico de deficiencias congénitas y adquiridas de factores de coagulación de la vía extrínseca (factores II, V, VII y X)[1,2]. Se usa también para la inducción y monitorización del tratamiento anticoagulante oral[3,4] y puede usarse para valorar la capacidad de síntesis de proteínas del hígado en trastornos hepáticos crónicos o agudos.
La Thromboplastin L tiene su origen en cerebro de conejo, pero se parece a la BCT humana en su bajo Índice de Sensibilidad Internacional (ISI). El ISI de Thromboplastin L es de aproximadamente 1,1 y se calibra contra el preparado de referencia internacional de la OMS[5]. La prueba de Thromboplastin L está especialmente adaptada a la monitorización del tratamiento anticoagulante oral y, conjuntamente con el plasma deficiente en el factor oportuno, la medición de la actividad de los factores en la vía extrínseca. La tromboplastina tisular, en presencia de iones calcio, es un activador que inicia la vía extrínseca de la coagulación. Cuando se añade una mezcla de tromboplastina tisular e iones calcio al plasma normal citratado, se activa el mecanismo de coagulación, conduciendo a un coágulo de fibrina. Si se produce una deficiencia dentro de la vía extrínseca, el tiempo necesario para la formación de coágulos se prolongará dependiendo de la intensidad de la deficiencia.

ADVERTENCIAS Y PRECAUCIONES

Los reactivos que contiene este kit son sólo para uso de diagnóstico *in vitro*: NO INGERIR. Lleve el equipo de protección personal adecuado cuando utilice todos los componentes del kit. Consulte la declaración de seguridad del producto para saber más sobre las indicaciones adecuadas de advertencia y riesgo. Desechar los componentes de conformidad con las normativas locales.

COMPOSICIÓN

Componente	Contiene	Descripción	Preparación
Thromboplastin L	2 x 5 mL (REF 5265HL) 8 x 5 mL (REF 5265L) 10 x 10 mL (REF 5267L)	Tromboplastina líquida de cerebro de conejo que contiene cloruro de calcio, estabilizadores y conservantes.	La tromboplastina líquida calcificada está lista para su uso. No es necesario más calcio para realizar pruebas estándar de PT. Los contenidos del vial se deben mezclar bien antes de utilizarlo (5 minutos en el rodillo).

Cada kit contiene instrucciones de uso.

Cada kit contiene valores de referencia específicos insertados del lote.

ARTÍCULOS NECESARIOS NO SUMINISTRADOS

Los siguientes productos se puede utilizar junto con la Thromboplastin L:

REF 5519	ISI Calibrant Plasma Set
REF 5490	INR Reference Set

ALMACENAMIENTO, CADUCIDAD Y ESTABILIDAD

Los reactivos no abiertos son estables hasta la fecha de caducidad indicada cuando se conservan en las condiciones indicadas en el vial o en la etiqueta del kit.

Thromboplastin L — Los viales abiertos permanecen estables durante 2 meses a una temperatura de entre $^{+}$2 – $^{+}$8°C, 5 días a una temperatura mayor a $^{+}$15°C (en el Sysmex CA1500), y 6 horas a una temperatura mayor a $^{+}$37°C (en el AC-4, incluido el contenedor del reactivo y el tapón). Se puede conseguir estabilidad multiuso de 7 días (Sysmex CA-1500).
NO CONGELAR. Los grumos grandes de partículas o los cambios en los valores esperados pueden indicar deterioro del producto.

RECOGIDA Y PREPARACIÓN DE LAS MUESTRAS

Debe usarse siempre plástico o vidrio siliconizado. Debe recogerse sangre (9 partes) en el anticoagulante citrato sódico al 3,2% o al 3,8% (1 parte). Separar el plasma después de la centrifugación a 1500 x g durante 15 minutos. El plasma debe conservarse a $^{+}$18 –$^{+}$24°C. Las pruebas deberían terminarse en 4 horas desde la recogida de las muestras o el plasma puede conservarse congelado a -20°C durante 2 semanas o -70°C durante 6 mes. Descongelar rápidamente a $^{+}$37°C antes de realizar la prueba. No conservar a $^{+}$37°C durante más de 5 minutos[6].

PROCEDIMIENTO

Para la comunicación exacta del INR, se recomienda determinar la ISI específica del laboratorio del reactivo con el sistema de prueba en uso. Se recomienda el ISI Calibrant Plasma Set de Helena Biosciences Europe (REF 5519) para este fin[7,8]. Esto debe realizarse para cada nuevo lote de reactivos. Debe usarse el Set de Referencia de INR de Helena Biosciences Europe (REF 5490) para comprobar las desviaciones en el ISI del sistema local que se han observado con cambios en la temperatura del laboratorio y el mantenimiento posinstrumental, entre otras variaciones locales.

Método Manual

1. Mezclar la cantidad suficiente de Thromboplastin L para completar la prueba anticipada para el día e incubar a +37°C durante un período de tiempo no superior a 4 horas.
2. Precaliente 0,1 mL del plasma de prueba a $^{+}$37°C durante 2 minutos.
3. Añada 0,2 mL de reactivo de tromboplastina recién mezclado al plasma mientras pone en marcha simultáneamente un cronómetro.
4. observa el tiempo hasta la formación del coágulo procurando afinar en la décima 0,1 de segundo más próxima.

Método Automatizado

Consulte el manual del usuario del instrumento adecuado para instrucciones detalladas o póngase en contacto con Helena Biosciences Europe para notas de aplicación específicas del instrumento.

INTERPRETACIÓN DE LOS RESULTADOS

Los resultados deben comunicarse en los 0,1 segundos más próximos y las pruebas duplicadas deben estar de acuerdo entre sí dentro del 5%. Los valores de %TP pueden interpolarse a partir del gráfico de calibración (%TP de los plasmas de calibración del TP frente al tiempo de coágulo medido) que debe ser una línea recta cuando se representa en un papel de gráficos log-log.

Los valores de INR pueden calcularse usando la siguiente fórmula: INR = (Tiempo de TP Paciente / Tiempo de TP normal medio)^{ISI}

Si necesita una guía clara de indicaciones y tratamiento de pacientes con warfarina, consulte The British Society for Haematology y su última edición de "Guidelines on oral anticoagulation with warfarin" que, en el momento de impresión de este documento, se encuentra en su cuarta edición de 2011[9].

LIMITACIONES

El uso de diluciones seriadas de un plasma de referencia para la curva de %TP no se recomienda, porque puede llevar a discrepancias producidas por el bajo fibrinógeno en las diluciones del plasma de referencia, que no se reflejan en las muestras de pacientes que tienen fundamentalmente niveles normales de fibrinógeno. Helena Biosciences Europe recomienda utilizar el kit 5504R %PT/INR directa a tal fin.

CONTROL DE CALIDAD

Cada laboratorio debe establecer un programa de control de calidad. Los controles normales y anormales deben estudiarse antes de cada lote de muestras del paciente, para asegurar un funcionamiento adecuado del instrumento y el operador. Si los controles no se realizan como se esperaba, los resultados del paciente deben considerarse inválidos.
Helena Biosciences Europe suministra los siguientes controles disponibles para usar con este producto:

REF 5186	Routine Control N
REF 5187	Routine Control A
REF 5183	Routine Control SA
REF 5490	INR Reference Set

VALORES DE REFERENCIA

Los valores de referencia pueden variar entre los laboratorios dependiendo de las técnicas y sistemas usados. Por esta razón, cada laboratorio debe establecer su propio intervalo normal. Esto es especialmente importante para la calibración local del ISI. Con la serie de instrumentos Sysmex, es frecuente que los valores normales varíen entre 11,50 - 14,60 segundos; 0.930 - 1.160 INR; 79.10 - 112.80 %PT .

CARACTERÍSTICAS FUNCIONALES

Las siguientes características de rendimiento han sido determinadas por Helena Biosciences Europe o sus representantes usando un instrumento de coagulación Sysmex CA-1500. Cada laboratorio debe establecer sus propios datos de rendimiento.

Reproductibilidad

Muestra	Routine Control N		Routine Control A		Routine Control SA	
	SD	*CV (%)*	*SD*	*CV (%)*	*SD*	*CV (%)*
Repetibilidad	0,07	0,59	0,24	1,09	0,45	1,11
Entre series	0,10	0,83	0,16	0,75	0,49	1,20
Entre días	0,04	0,32	0,06	0,27	0,25	0,62
En el dispositivo/laboratorio	0,12	1,07	0,29	1,35	0,72	1,75

Interferencias

Helena Thromboplastin L no detecta niveles de heparina inferiores a 2 U/mL.Con un umbral de interferencia del 5%, no hay interferencias significativas de hemoglobina en concentraciones de hasta 10 g/L. Con un umbral de interferencia del 5%, no hay interferencias significativas de bilirrubina en concentraciones de hasta 0,5 g/L para Thromboplastin L. La interferencia de lípidos demuestra que los niveles de lípidos no afectan directamente al tiempo de coagulación del reactivo hasta 3,75 g/L. Las concentraciones de lípidos superiores evitan detectar la coagulación.

Comparación del método

La comparación del tiempo de coagulación y los valores INR se determinó con tromboplastina L y tromboplastina LI en 268 muestras. Se obtuvieron las siguientes correlaciones:

Thromboplastin L (segundo) = 0,9911x + 0,1038	r^2 = 0,9941	n = 268
Thromboplastin L (INR) = 0,9853x + 0,0261	r^2 = 0,9500	n = 268

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Тест-система "Жидкий тромбопластин"
инструкция

ru

НАЗНАЧЕНИЕ

Комплект Тест-система "Жидкий тромбопластин" предназначен для выполнения анализов гемостаза на основе кровяного сгустка.

Впервые стандартизированный одностадийный тест для определения протромбинового времени был предложен д-ром Армандом Квиком в 1935г. Сейчас он стал основным скрининговым тестом для диагностики врожденного и приобретенного дефицитов факторов свертывания крови по внешнему пути (факторы II, V, VII и X)[1,2]. Он также используется для мониторинга терапии оральными антикоагулянтами[3,4], а также может применяться и для оценки белок синтетической функции печени при ее острых либо хронических заболеваниях.
Реагент приготовлен из экстракта ткани мозга кролика и по значению своего МИЧ (Международного Индекса Чувствительности) сопоставим со Стандартом Тромбопластина Британии (BCT – British Comparative Thromboplastin). МИЧ Жидкого тромбопластина примерно равен 1.1 и откалиброван относительно международного референса BO3[5]. Жидкий тромбопластин особенно подходит для мониторинга терапии оральными антикоагулянтами и для измерения активности факторов внешнего пути свертывания(вместе с соответствующей фактор-дефицитной плазмой). Тканевый тромбопластин в присутствии йонов кальция является активатором внешнего пути свертывания крови. При добавлении смеси тканевого тромбопластина с кальцием к нормальной цитратной плазме активируется механизм её свертывания, приводящий к образованию фибринового сгустка. Если у пациента имеет место дефицит факторов внешнего пути, то время, необходимое для образования сгустка будет удлиняться прямо пропорционально степени дефицита факторов.

ПРЕДУПРЕЖДЕНИЯ И МЕРЫ ПРЕДОСТОРОЖНОСТИ

Содержащиеся в данном наборе реагенты предназначены только для *in vitro* диагностики– НЕ ПРИНИМАТЬ ВНУТРЬ! При работе со всеми компонентами набора использовать соответствующие средства индивидуальной защиты. В случае необходимости см. свидетельство о безопасности изделия для ознакомления с соответствующими описаниями опасного воздействия и сведениями о мерах предосторожности. Удаление компонентов в отходы производите в соответствии с местными правилами.

СОСТАВ

Компоненты	Состав набора	Описание	Приготовление реагентов
Жидкий тромбопластин	2 x 5 мл (Кат.№ 5265HL) 8 x 5 мл (Кат.№ 5265L) 10 x 10 мл (Кат.№ 5267L)	Тромбопластин-кальциевая суспензию реагента из экстракта ткани мозга кролика, раствора хлорида кальция и стабилизаторов	Жидкий кальцинированный тромбопластин, готовый для использования. Для проведения теста ПВ дополнительный кальций не требуется. Содержимое флакона перемешать до использования (5 мин. на роллере)

Каждый набор содержит инструкцию по применению.

Вкладыш с указанием референсных значений к различным анализаторам и методам исследования, характерным для данного лота.

НЕОБХОДИМЫЕ КОМПОНЕНТЫ, НЕ ВКЛЮЧЕННЫЕ В КОМПЛЕКТ ПОСТАВКИ

Продукция ниже, которая может использоваться вместе с Тест-системой «Тромбопластин»:

Кат.№ 5519	Калибратор МИЧ
Кат.№ 5490	Контроль на МНО

ХРАНЕНИЕ, СРОК ГОДНОСТИ И УСТОЙЧИВОСТЬ

Невскрытые флаконы с реагентами хранятся до истечения срока годности в условиях, указанных на этикетке.

Жидкий тромбопластин — Вскрытые флаконы годны к применению в течение 2 месяцев при температуре хранения 2-8°C, 5 дней при 15°C (на борту Sysmex CA-1500) и 6 часов при 37°C (на борту AC-4, используя контейнер для реагентов с крышкой). Стабильность реагента на борту Sysmex CA-1500 - 7 дней.
НЕ ЗАМОРАЖИВАТЬ! Признаки ухудшения качества реагента: большое скопление частиц или отличия от ожидаемых значений могут означать ухудшение свойств реактива.

ОТБОР И ПОДГОТОВКА ОБРАЗЦОВ

Для работы следует использовать только пластиковые или силиконированные стеклянные пробирки. Кровь забирается в пробирку с цитратным антикоагулянтом (3,2% или 3,8% цитрат натрия) в соотношении 9 + 1. После центрифугирования при 1500 g, в течение 15 минут (использование других параметров должно проверяться лабораторией), полученную плазму необходимо отделить от форменных элементов крови. Плазму следует хранить при температуре $^{+}$18 –$^{+}$24°C. Тестирование должно быть проведено в течение 4 часов после забора образцов, либо плазму можно однократно заморозить и хранить при температуре -20°C в течение 2 недель или при -70°C в течение 6 месяцев. Перед проведением исследования плазму следует быстро разморозить при $^{+}$37°C. Не держать плазму при $^{+}$37°C более 5 минут[6].

ПРОЦЕДУРА

Для установления более точного МНО рекомендуется определить специфическое для лаборатории значение МИЧ (так называемый «локальный» МИЧ) для жидкого тромбопластина. Helena Biosciences Europe рекомендует для этих целей применять Калибратор МИЧ (Кат.№ REF 5519)[7,8]. Подобная процедура должна быть проведена для каждой новой партии реагентов. Контроль на МНО (Кат.№ REF 5490) используется для контроля изменения в «локальном» МИЧ, так как его значение может незначительно варьировать в зависимости от используемых приборов, а также изменений температуры в лаборатории и других локальных факторов.

Ручной Метод

1. Взять необходимое количество жидкого тромбопластина, необходимое для проведения тестирования в течение дня и инкубировать при 370С (но не более 4 часов).
2. Прогреть 0,1 мл тестируемой плазмы при $^{+}$37°C в течение 2 минут.
3. Добавить 0,2 мл свежеприготовленной рабочей смеси реагентов к плазме и немедленно включить таймер.
4. Заметить время, необходимое для образования сгустка с точностью до 0,1сек.

Автоматизированный Метод

Для проведения тестирования на автоматических коагулометрах, необходимо обратиться к руководству пользователя и к соответствующей адаптации, которые доступны у официальных дистрибьюторов и компании Хелена.

ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Результаты должны быть представлены с точностью до 0,1 сек и разница в повторах не должна превышать 5%. Значение %ПВ можно определить по калибровочному графику (график зависимости %ПВ от измеренного времени), который должен быть строго линейным в билогарифмических координатах при построении графика на миллиметровке с логарифмическим масштабом на обеих осях.

МНО может быть вычислено по формуле: МНО= {ПВ (пациента)/среднее ПВ (норма)}^{МИЧ}

Для более подробной информации по мониторингу пациентов на Варфарине обратитесь к рекомендациям Британского Общества по Гематологии - «Рекомендации по антикоагулянтной терапии варфарином», 4-е издание 2011г. на время печати этого документа[9].

ОГРАНИЧЕНИЯ

Для получения графика зависимости (%ПТ) от времени свертывания не рекомендуется использовать серии разведений референсной плазмы, поскольку это может привести к неточности, вызванной низким содержанием фибриногена в больших разведениях референсной плазмы, что не соответствует образцам пациентов, имеющих, в основном, нормальный уровень фибриногена. Компания Хелена рекомендует использовать калибратор кат. номер 5504R для %ПВ, и прямого определения МНО.

КОНТРОЛЬ КАЧЕСТВА

Каждая лаборатория должна установить программу контроля качества. Перед измерением каждой партии образцов пациентов необходимо протестировать нормальную и патологическую плазму, чтобы удостовериться в удовлетворительной работе оборудования и оператора. Если контрольные измерения не совпадают с ожидаемыми значениями, то измеренные данные пациентов следует считать недостоверными.
Компания Хелена рекомендует следующие контрольные материалы:

Кат.№ 5186	Контроль качества, норма
Кат.№ 5187	Контроль качества, умеренно выраженная патология
Кат.№ 5183	Контроль качества, высокая патология
Кат.№ 5490	Контроль на МНО

НОРМАЛЬНЫЕ ПОКАЗАТЕЛИ

Референсные значения могут варьировать между лабораториями в зависимости от используемых методов и анализаторов. В связи с этим каждая лаборатория должна определить свою собственную область нормальных значений. Это крайне важно для установки локального МИЧ тромбопластина. Диапазон нормальных значений пациентов 11,50 - 14,60 сек.; 0.930 - 1.160 INR; 79.10 - 112.80 %PT с использованием коагулометров Sysmex.

ЭКСПЛУАТАЦИОННЫЕ ХАРАКТЕРИСТИКИ

Компания Хелена или её дистрибьюторы определили следующие ориентировочные аналитические характеристики. Каждая лаборатория должна определить свои собственные аналитические характеристики. При использовании оптико-механического коагулометра и реагентов Хелена были определены следующие коэффициенты вариации (CV):

Воспроизводимость

Образец	Контроль качества, норма		Контроль качества, умеренно выраженная патология		Контроль качества, высокая патология	
	SD	*CV (%)*	*SD*	*CV (%)*	*SD*	*CV (%)*
Воспроизводимость	0,07	0,59	0,24	1,09	0,45	1,11
в пределах аналитической серии	0,10	0,83	0,16	0,75	0,49	1,20
между разными аналитическими сериями	0,04	0,32	0,06	0,27	0,25	0,62
между партиями/лабораториями	0,12	1,07	0,29	1,35	0,72	1,75

Тест-система "Жидкий тромбопластин" не чувствителен к гепарину в концентрации до 2 Ед/мл. Используя 5% допустимое отклонение, реагент не чувствителен к гемоглобину в концентрации до 10 г/Л. Используя 5% допустимое отклонение, реагент не чувствителен к билирубину в концентрации до 0.5 г/Л. Не обнаружено чувствительности к уровню липидов до 3.75 г/Л оказывающих воздействие на время свёртываемости. Концентрации липидов выше этого уровня предотвратит обнаружение сгустка.

Сравнение со стандартами:
Сравнение значений МНО 268 образцов, определённых с помощью Тест-системы "Тромбопластин" и Тест-системы "Жидкий тромбопластин" дало следующие корреляционные соотношения:

Жидкий Тромбопластин (сек) = 0,9911x + 0,1038	r^2 = 0,9941	n = 268
Жидкий Тромбопластин (МНО) = 0,9853x + 0,0261	r^2 = 0,9500	n = 268

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Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Anti-Thyroid Peroxidase (Anti-TPO) Test System

Product Code: 1125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroid Peroxidase (TPO) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric.
Measurements of TPO autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goiter.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Antibodies to thyroid peroxidase have been shown to be characteristically present from patients with Hashimoto thyroiditis (95%), idiopathic myedema (90%) and Graves Disease (80%)[1]. In fact 72% of patients positive for anti-TPO exhibit some degree of thyroid dysfunction.[2] This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction.

Measurements of antibodies to TPO have been done in the past by Passive Hemaglutination (PHA). PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits the detectability of subclinical levels of antibodies to TPO. In addition, the results are quantitated by a spectrophotometer, which eliminates subjective interpretation.

Monobind's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated Thyroid Peroxidase Antigen (TPO) is added, and then the reactants are mixed. Reaction results between the autoantibodies to TPO and the biotinylated TPO to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.

After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce color.

The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen's enzyme activity can be correlated with autoimmune antibody level.

3.0 PRINCIPLE

A Sequential ELISA Method (TYPE 1)
The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated thyroid peroxidase antigen.

Upon mixing the biotinylated antigen and a serum containing the autoantibody, a reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$h\text{-}Ab_{(X\text{-}TPO)} + {}^{Btn}Ag_{(TPO)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} h\text{-}Ab_{(X\text{-}TPO)} - {}^{Btn}Ag_{(TPO)}$$

$^{Btn}Ag_{(TPO)}$ = Biotinylated Antigen (Constant Quantity)
$h\text{-}Ab_{(X\text{-}TPO)}$ = Human Auto-Antibody (Variable Quantity)
$Ab_{(X\text{-}TPO)} - {}^{Btn}Ag_{(TPO)}$ = Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:

$h\text{-}Ab_{(X\text{-}TPO)} - {}^{Btn}Ag_{(TPO)} + \underline{\text{Streptavidin}}_{C.W.} \Rightarrow$ Immobilized complex (IC)

$\underline{\text{Streptavidin}}_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex (IC) = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.

$I.C._{(h\text{-}IgG)} + {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} \Rightarrow {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$

$I.C._{(h\text{-}IgG)}$ = Immobilized Immune complex (Variable Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG}$ = Enzyme-antibody Conjugate (Constant Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG} - I.C._{(h\text{-}IgG)}$ = Ag-Ab Complex (Variable Quantity)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained

4.0 REAGENTS

Materials Provided

A. Anti-TPO Calibrators – 1ml/vial Icons A-F
Six (6) vials of references for anti-TPO at levels of 0(A), 25(B), 50(C), 100(D), 250(E) and 500(F) IU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the Medical Research Council (MRC) International Standard 66/387 for anti thyroid microsome.

B. TPO Biotin Reagent – 13ml/vial – Icon ▽
One (1) vial of biotinylated thyroid peroxidase antigen stabilized in a buffering matrix. A preservative has been added. Store at 2-8°C

C. Anti-TPO Enzyme Reagent – 13ml/vial - Icon
One (1) vial of anti-human IgG-horseradish peroxidase (HRP) conjugate stabilized in a bufferred matrix. A preservative has been added. Store at 2-8°C

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Serum Diluent – 20ml/vial
One (1) vial of serum diluent concentrate that containing buffer salts and a dye. Store at 2-8°C.

F. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

G. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

H. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See "Reagent Preparation."

I. Stop Solution – 8ml/vial - Icon
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

J. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

Required But Not Provided:
1. Pipette capable of delivering 0.010ml (10µl), 0.025ml (25µl), and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Test tube(s) for patient dilution.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8ºC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the samples(s) may be stored at temperatures of -20ºC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Serum Diluent**
   Dilute the serum diluent to 200ml in a suitable container with distilled or deionized water. Store at 2-8°C.
2. **Wash Buffer**
   Dilute contents of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
3. **Working Substrate Solution** – Stable for one (1) year.
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.
4. **Patient Sample Dilution (1/100)**
   Dispense 0.010ml (10µl) of each patient specimen into 1ml (1000µl) of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note1 : Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or diluted patient specimen into the assigned well.
3. Add 0.100 ml (100µl) of the TPO Biotin Reagent
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of the x-TPO Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Incubate for thirty (30) minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 350µl of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
    DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 500 IU/ml, dilute the sample an additional 1:5 or 1:10 using the original diluted material. Multiply by the dilution factor to obtain the concentration of the specimen.

10.0 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-TPO in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-TPO activity in IU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the level of anti-TPO activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.323) intersects the dose response curve at 200 IU/ml anti-TPO concentration (See Figure 1).

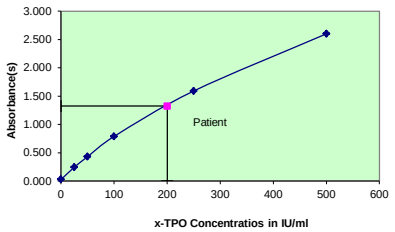
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (IU/ml)
Cal A	A1	0.022	0.026	0
	B1	0.030		
Cal B	C1	0.240	0.244	25
	D1	0.247		
Cal C	E1	0.437	0.430	50
	F1	0.422		
Cal D	G1	0.795	0.788	100
	H1	0.782		
Cal E	A2	1.610	1.590	250
	B2	1.572		
Cal F	C2	2.659	2.600	500
	D2	2.533		
Patient	E2	1.294	1.323	**200**
	F2	1.351		

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Very high concentration of anti-TPO in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

The presence of autoantibodies to TPO is confirmed when the serum level exceeds 40 IU/ml. The clinical significance of the result, coupled with anti-thyroglobulin activity, should be used in evaluating the thyroid condition. However, clinical inferences should not be solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests.

13.0 EXPECTED RANGES OF VALUES

A study of normal population was undertaken to determine expected values for the anti-TPO AccuBind® ELISA test system. The number (n), mean (x) and standard deviation (σ) are given in Table 1. Values in excess of 40IU/ml are considered positive for the presence of anti-TPO autoantibodies.

TABLE I
Expected Values for the Anti-TPO ELISA Test System (In IU/ml)

Number	**100**
Mean	17.6
Standard deviation	10.8
Upper 95% (+2σ) level	39.2

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the anti-TPO AccuBind® ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V) for each of these control sera are presented in Tables 2 and 3.

TABLE 2
Within Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	20	25.5	1.5	5.7%
Pool 2	20	120.5	4.6	3.8%
Pool 3	20	352.4	14.8	4.2%

TABLE 3*
Between Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	10	26.5	1.8	6.8%
Pool 2	10	118.5	5.3	4.5%
Pool 3	10	365.4	22.5	6.2%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The anti-TPO AccuBind® ELISA test system has a sensitivity of 0.92 IU/ml. The sensitivity (detection limit) was ascertained by determining the variablility of the '0 IU/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The anti-TPO AccuBind® ELISA test system was compared with a reference anti-TPO ELISA microplate. Biological specimens from normal and disease states populations were used. The disease states included; Hashimoto's thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 82. The least square regression equation and the correlation coefficient were computed for the anti-TPO AccuBind® ELISA test system in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	122.9	y = 1.02 (x) – 5.1	0.989
Reference	127.0		

14.4 Specificity

Interferences from ANA, DNA, thyroglobulin (TPO) and rheumatoid antibodies were found to be insignificant

15.0 REFERENCES

1. Volpé R, "Autoimmune disease of the endocrine system", Boca Raton FL, *CRC Press* (1990).
2. Volpé R, *Clin Chem*, 40, 2132 (1994).
3. Beever K, et al, *Clin Chem*, 35, 1949-54 (1989).
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5. Czarnocka B, Ruff J, Ferrand M, Carayon P, Lissitzky S, "Purification of the human thyroid and its identification as the microsomal antigen involved in the human thyroid disease", *FEBS Letts*, 190, 147-52 (1985).
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8. Nunez J. Pommier J, "Formation of thyroid hormones", *Vitam Horm,* 39, 175-229 (1982).
9. Ekholm R, "Biosysnthesis of thyroid hormones", *Int Rev Cytol,* 120, 243-288 (1990).
10. Degroot LJ, "Heterogeneity of human antibodies to TPO Thyroperoxidase", *Thyroid Autoimmunity,* **207,**177-182 (1990).

Revision: 4 Date: 2019-JUL-16 DCO: 1353
MP1125 Product Code: *1125-300*

Size		96(A)
Reagent (fill)	A)	1ml set
	B)	1 (13ml)
	C)	1 (13ml)
	D)	1 plate
	E)	1 (20ml)
	F)	1 (20ml)
	G)	1 (7ml)
	H)	1 (7ml)
	I)	1(8ml)

For Orders and Inquires, please contact
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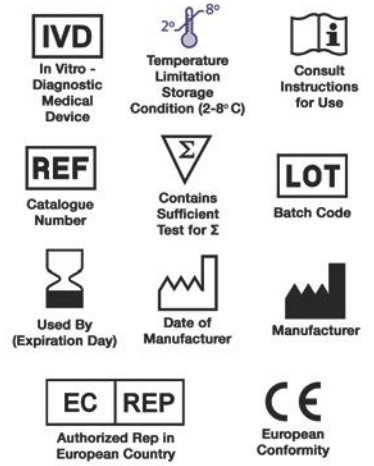
Glossary of Symbols
(EN 980/ISO 15223)



Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Thyroglobulin Ab (Anti-Tg) Test System
Product Code: 1025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroglobulin (Tg) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric. Measurements of Tg autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goiter.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Antibodies to thyroglobulin have been shown to be characteristically present from patients with thyroiditis and primary thyrotoxicosis.[1,2] This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction. Passive Hemaglutination (PHA) methods have been employed in the past for measurements of antibodies to Tg. PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits the detectability of subclinical levels of antibodies to Tg. In addition, the results are quantitated by a spectrophotometer, which eliminates subjective interpretation.

Monobind's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated thyroglobulin (Tg) is added, and then the reactants are mixed. Reaction results between the autoantibodies to Tg and the biotinylated Tg to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.

After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce color.

The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen's enzyme activity can be correlated with autoimmune antibody level.

3.0 PRINCIPLE

A Sequential Sandwich ELISA Method (TYPE 1)
The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated thyroglobulin antigen.

Upon mixing biotinylated antigen and a serum containing the autoantibody, reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$\text{h-Ab}_{(X\text{-}Tg)} + {}^{Btn}\text{Ag}_{(Tg)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} \text{h-Ab}_{(X\text{-}Tg)} - {}^{Btn}\text{Ag}_{(Tg)}$$

$^{Btn}Ag_{(Tg)}$ = Biotinylated Antigen (Constant Quantity)
$h\text{-}Ab_{(X\text{-}Tg)}$ = Human Auto-Antibody (Variable Quantity)
$Ab_{(X\text{-}Tg)} - {}^{Btn}Ag_{(Tg)}$ = Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:
$h\text{-}Ab_{(X\text{-}Tg)} - {}^{Btn}Ag_{(Tg)} + \underline{\text{Streptavidin}}_{C.W.} \Rightarrow \underline{\text{immobilized complex}}$ (IC)
$\underline{\text{Streptavidin}}_{C.W.}$ = Streptavidin immobolized on well
$\underline{\text{Immobilized complex (IC)}}$ = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.
$I.C._{(h\text{-}IgG)} + {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} \Rightarrow {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$
$I.C._{(h\text{-}IgG)}$ = Immobilized Immune complex (Variable Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG}$ = Enzyme-antibody Conjugate (Constant Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$ = Ag-Ab Complex (Variable Quantity)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Anti-Tg Calibrators – 1ml/vial Icons A-F
Six (6) vials of references for anti-Tg at levels of 0(**A**), 50(**B**), 125(**C**), 500(**D**), 1000(**E**), and 2000(**F**) IU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using the 1st International Reference Preparation, which was assayed against the Medical Research Council (MRC) Research Standard A 65/93 for anti-thyroglobulin activity.

B. Tg Biotin Reagent – 13ml/vial – Icon ∇
One (1) vial of biotinylated thyroglobulin stabilized in a buffering matrix. A preservative has been added. Store at 2-8°C**.**

C. x-Tg Enzyme Reagent – 13ml/vial - Icon
One (1) vial of anti-human IgG-horseradish peroxidase (HRP) conjugate stabilized in a bufferred matrix. A preservative has been added. Store at 2-8°C**.**

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Serum Diluent – 20ml/vial
One (1) vial of serum diluent concentrate that containing buffer salts and a dye. Store at 2-8°C.

F. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

G. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

H. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See "Reagent Preparation."

I. Stop Solution – 8ml/vial - Icon
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

J. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.0101ml (10.1µl) and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Test tube(s) for patient dilution.
9. Timer.
10. Quality control materials.

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All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or plasma in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

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Dilute contents of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
3. **Working Substrate Solution** – Stable for one (1) year.
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.
4. **Patient Sample Dilution (1/100)**
Dispense 0.0101ml (10.1µl) of each patient specimen into 1ml (1000µl) of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

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*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or diluted patient specimen into the assigned well.
3. Add 0.100 ml (100µl) of Tg Biotin Reagent.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of x-Tg Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITON
9. Cover and incubate for thirty (30) minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of Working Substrate Solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITON
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 2000 IU/ml, dilute the sample an additional 1:5 or 1:10 using

the original diluted material. Multiply by the dilution factor to obtain the concentration of the specimen.

10.0 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-Tg in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-Tg activity in IU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the level of anti-Tg activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.387) intersects the dose response curve at 790 IU/ml anti-Tg concentration (See Figure 1).

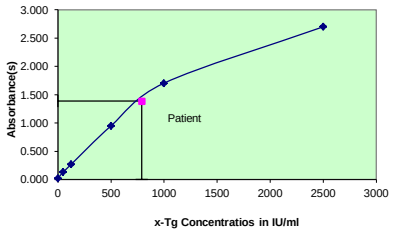
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (IU/ml)
Cal A	A1	0.022	0.025	0
	B1	0.028		
Cal B	C1	0.135	0.133	50
	D1	0.131		
Cal C	E1	0.280	0.270	125
	F1	0.261		
Cal D	G1	0.962	0.949	500
	H1	0.936		
Cal E	A2	1.709	1.703	1000
	B2	1.698		
Cal F	C2	2.730	2.698	2000
	D2	2.667		
Patient	E2	1.390	1.387	790
	F2	1.383		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 'F' should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Very high concentration of anti-Tg in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
10. Samples, which are contaminated microbiologically, should not be used.
11. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
12. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
13. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
14. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. The presence of autoantibodies to Tg is confirmed when the serum level exceeds 125 IU/ml. The clinical significance of the result, coupled with anti-thyroid peroxidase activity, should be used in evaluating the thyroid condition. However, clinical inferences should not be solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests.
8. The cost benefits should be considered in the use of thyroglobulin antibodies testing when performed in concert with anti- thyroid peroxidase (TPO). The widespread practice of performing both tests has been questioned.[4]

13.0 EXPECTED RANGES OF VALUES

A study of normal population was undertaken to determine expected values for the Anti-Tg AccuBind® test system. The number (n) mean (X) and standard deviation (σ) are given in Table 1. Values in excess of 125IU/ml are considered positive for the presence of anti-Tg autoantibodies.

TABLE I
Expected Values for Anti-Tg AccuBind® ELISA Test System (In IU/ml)

Number	**100**
Mean	74.3
Standard deviation	25.2
Upper 95% (+2σ) level	124.7

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the Anti-Tg AccuBind® ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value(X), standard deviation (σ) and coefficient of variation (C.V) for each of these control sera are presented in Tables 2 and 3.

TABLE 2
Within Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	20	65.5	3.3	5.0%
Pool 2	20	385.5	15.5	4.0%
Pool 3	20	1554.4	55.4	3.6%

TABLE 3*
Between Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	10	66.8	3.6	5.3%
Pool 2	10	374.2	18.5	4.9%
Pool 3	10	1625.5	65.2	4.0%

*As measured in ten experiments in duplicate.

12.2Sensitivity

The Anti-Tg AccuBind® ELISA has a sensitivity of 1.94 IU/ml. The sensitivity was ascertained by determining the variability of the '0 IU/ml' calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

12.3 Accuracy

The Anti-Tg AccuBind® ELISA test system was compared with a reference method. Biological specimens from normals, and disease states populations were used. The disease states included; Hashimoto's thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 181. The least square regression equation and the correlation coefficient were computed for the anti-Tg AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	415.6	y = 9.79 + 0.969 (x)	0.995
Reference	419.2		

Only slight amounts of bias between the anti-Tg AccuBind® ELISA method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

Interferences from ANA, DNA, thyroid peroxidase (TPO) and rheumatoid antibodies were found to be insignificant in the assay system.

15.0 REFERENCES

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9. Ekholm R, "Biosysnthesis of thyroid hormones*", Int Rev Cytol,* 120, 243-288 (1990).
10. Degroot LJ, "Heterogentiy of human antibodies to TPO Thyroperoxidase", *Thyroid Autoimmunity,* 207, 177-182 (1990).

Revision: 5 Date: 2019-JUL-16 DCO: 1353
MP1025 Product Code: *1025-300*

Size		96(A)
Reagent (fill)	A)	1ml set
	B)	1 (13ml)
	C)	1 (13ml)
	D)	1 plate
	E)	1 (20ml)
	F)	1 (20ml)
	G)	1 (7ml)
	H)	1 (7ml)
	I)	1(8ml)

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IVD 2° 8° CE

EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device

2° 8° — Temperature Limitation Storage Condition (2-8°C)

Consult Instructions for Use

REF — Catalogue Number

Σ — Contains Sufficient Test for Σ

LOT — Batch Code

Used By (Expiration Day)

Date of Manufacturer

Manufacturer

EC REP — Authorized Rep in European Country

CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Cancer Antigen 125 (CA-125) Test System

Product Code: 3025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Cancer Antigen 125 (CA-125) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Cancer Antigen 125 (CA-125) is a glycoprotein that occurs in blood as high molecular weight entity (MB_{rB} > *200*,000). High concentrations of this antigen are associated with ovarian cancer and a range of benign and malignant diseases. Although the specificity and sensitivity of CA-125 assays are somewhat limited, especially in early diagnosis of ovarian cancer, the assay has found widespread use in the differential diagnosis of adnexal masses, in monitoring disease progression and response to therapy in ovarian cancer, and in the early detection of recurrence after surgery or chemotherapy for ovarian cancer. Published literature has shown that elevated serum CA-125 levels can be observed in patients with serious endometroid, clear cell and undifferentiated ovarian carcinoma. The serum CA-125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, and 6% of patients with non-neoplastic conditions (including, but not limited to, first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum or pericardium).

In this method, CA-125 calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of CA-125) are added and the reactants mixed. Reaction between the various CA-125 antibodies and native CA-125 forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-CA-125 antibody bound conjugate is separated from the unbound enzyme-CA-125 conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of CA-125 levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with CA-125 concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA-125 antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{CA\text{-}125} + {}^{Btn}Ab_{(m)} \underset{k\text{-}a}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{CA\text{-}125}$ = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)} + Streptavidin_{CW} \Rightarrow$ Immobilized complex
$Streptavidin_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. CA-125 Calibrators - 1ml/vial- Icons A-F
Six (6) vials of references CA-125 Antigen at levels of 0(**A**), 15(**B**), 50(**C**), 100(**D**), 200(**E**) and 400(**F**) U/ml. A preservative has been added. Store at 2-8°C.

Note: The human serum based standards were made using a >99% pure affinity purified preparation of CA-125. The preparation was calibrated against Centocor CA-125 IRMA test.

B. CA-125 Enzyme-Reagent – 13ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial - Icon S^{A}
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

F. Substrate B – 7ml/vial - Icon S^{B}
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See "Reagent Preparation."

G. Stop Solution – 8ml/vial - Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 & 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and elevated ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one (1) year
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the CA-125 Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

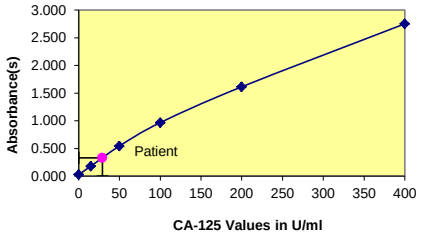
A dose response curve is used to ascertain the concentration of CA-125 in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CA-125 concentration in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of CA-125 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.331l) intersects the dose response curve at 29.3U/ml CA-125 concentration (See Figure 1).

Note: Computer data reduction software designed ELISA assays may also be used for the data reduction. *If such software is utilized, the validation of the software should be ascertained.*

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (U/ml)
Cal A	A1	0.035	0.029	0
	B1	0.022		
Cal B	C1	0.186	0.182	15
	D1	0.178		
Cal C	E1	0.536	0.545	50
	F1	0.554		
Cal D	G1	0.985	0.967	100
	H1	0.949		
Cal E	A2	1.615	1.615	200
	B2	1.616		
Cal F	C2	2.749	2.753	400
	D2	2.758		
Patient	A3	0.336	0.331	**29.3**
	B3	0.325		

Figure 1



*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with CA-125 concentrations above 400 U/ml may be diluted (for example 1/10 or higher) with normal male serum (CA-125 < 5 U/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
2. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. CA-125 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CA-125 value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.

13.0 EXPECTED RANGE OF VALUES

The serum CA-125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases and 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum or pericardium).

TABLE I
Expected Values for CA-125 AccuBind® ELISA Test System

Healthy and non-pregnant subjects	U≤U 35 U/ml

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the CA-125 AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	20	3.1	0.22	7.1%
Level 2	20	28.0	1.42	5.0%
Level 3	20	161.2	4.21	2.6%

TABLE 3
Between Assay Precision* (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	10	3.7	0.44	11.8%
Level 2	10	25.3	1.81	7.1%
Level 3	10	154.0	5.11	3.4%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CA-125 AccuBind® ELISA test system has a sensitivity of 1.0 U/ml. The sensitivity was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The CA-125 AccuBind® ELISA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 121. The least square regression equation and the correlation coefficient were computed for CA-125 in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (X)	5.67	y = -0.116 +1.032x	0.998
Reference (Y)	5.75		

14.4 Specificity

In order to test the specificity of the antibody pair used, massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. In addition some widely used, over-the-counter, drugs and some cytotoxic drugs (10 fold the normal dose) were tested in the assay. No cross reaction was found. Percent recoveries for some of these additions are listed below in Table 5.

TABLE 5

Analyte	Amount Added	% Recovery
Bilirubin	1 mMol/L	98 – 103%
Hemoglobin	1 mMol/L	100 – 106%
Triglycerides	10 mMol/L	96 – 110 %
RF	1000 kIU/L	97 – 107%
Biotin	25 µg/L	99 – 103%

15.0 REFERENCES

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Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP3025 Product Code: *3025-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
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www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device	2°-8° — Temperature Limitation Storage Condition (2-8°C)	Consult Instructions for Use
REF — Catalogue Number	Σ — Contains Sufficient Test for Σ	LOT — Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP — Authorized Rep in European Country	CE — European Conformity	

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Cancer Antigen 19-9 (CA 19-9) Test System

Product Code: 3925-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Cancer Antigen 19-9 (CA 19-9) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

A mucin type Sialyl Lewis Antigens group of glycoproteins (SLA) such as CA 19-9, 19-5 have been recognized as circulating cancer associated antigens for gastrointestinal cancer. The discovery of a monoclonal antibody clone (1116NS 19-9), which exhibited selective reactivity with human gastrointestinal carcinomas through the recognition of a carbohydrate determinant (CA 19-9) defined as a sialyl lacto-N-flucopenrose II, resulted in the successful purification and thus, determination of human gastrointestinal tumor associated glycoprotein antigen expressing CA 19-9 from colorectal carcinoma cell lines. Recently, reports indicate that serum CA 19-9 level is frequently elevated in the circulation of patients with various gastrointestinal malignancies, such as pancreatic, colorectal, gastric and hepatic carcinomas. Together with CEA, elevated CA 19-9 is suggestive of gallbladder disease. The tumor associated antigen may also be associated in some malignant conditions. Research studies demonstrate that serum CA 19-9 values may have utility in monitoring subjects with the above mentioned diagnosed malignancies.

In this method, CA 19-9 calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for CA 19-9) is added and the reactants mixed. Reaction between the CA 19-9 antibodies and native CA 19-9 forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. Another enzyme labeled monoclonal antibody specific to CA 19-9 is added to the wells. The enzyme labeled antibody binds to the CA 19-9 already immobilized on the well through its binding with the biotinylated monoclonal antibody. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the CA 19-9 in the sample.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA19-9 antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

$$Ag_{(CA19\text{-}9)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ag_{(CA19\text{-}9)} - {}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{(CA19\text{-}9)}$ = Native Antigen (Variable Quantity)
$Ag_{(CA19\text{-}9)}$ - ${}^{Btn}Ab_{(m)}$ = Antigen-antibody complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$Ag_{(CA19\text{-}9)}$ - ${}^{Btn}Ab_{(m)}$ + $Streptavidin_{CW} \Rightarrow$ Immobilized complex (IC)
$Streptavidin_{CW}$ = Streptavidin immobilized on well
Immobilized complex (IC) = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

$$(IC) + {}^{Enz}Ab_{(x\text{-}CA19\text{-}9)} \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(x\text{-}CA19\text{-}9)} - IC$$

${}^{Enz}Ab_{(x\text{-}CA19\text{-}9)}$ = Enzyme labeled Antibody (Excess Quantity)
${}^{Enz}Ab_{(x\text{-}CA19\text{-}9)}$ - IC = Antigen-Antibodies Complex
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

4.0 REAGENTS

Materials Provided:

A. CA 19-9 Calibrators – 1ml/vial - Icons A-F
Six (6) vials of human serum based reference calibrators at concentrations of 0 (**A**), 10 (**B**), 50 (**C**), 100 (**D**), 250 (**E**) and 500 (**F**) U/ml. A preservative has been added. Store at 2-8°C.
Note: The standards, human serum based, were made using a >99% pure affinity purified preparation of CA 19-9. The preparation was calibrated against Centocor CA 19-9 IRMA test.

B. CA 19-9 Biotin Reagent – 13ml/vial ∇
One (1) vial of Anti-Human CA19-9 (MoAb)-Biotin reagent in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. CA 19-9 Enzyme Reagent – 13ml/vial - Icon Ⓔ
One (1) vial of Anti-Human CA19-9-HRP conjugate in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

D. Streptavidin Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C. See "Reagent Preparation."

G. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in acetate buffer. Store at 2-8°C. See "Reagent Preparation."

H. Stop Solution – 8ml/vial - Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 & 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one (1) year
Pour the contents of vial labeled Solution 'A' into the vial labeled Solution 'B'. Place the yellow cap on the mixed reagent for easy identification. Mix and label accordingly. Store at 2-8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 - 27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the biotinylated labeled antibody to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see "Reagent Preparation"), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100ml (100µl) of the CA19-9 Enzyme Reagent labeled antibody to each well.
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Cover and incubate 60 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

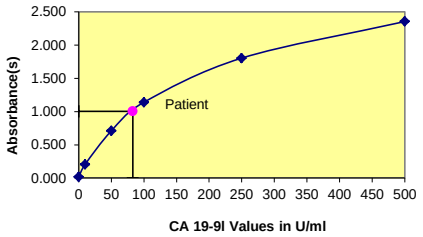
A dose response curve is used to ascertain the concentration of CA19-9 in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CA 19-9 concentration in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of CA 19-9 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.004) intersects the dose response curve at 82.9U/ml CA 19-9 concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (U/ml)
Cal A	A1	0.013	0.014	0
	B1	0.014		
Cal B	C1	0.210	0.208	10
	D1	0.212		
Cal C	E1	0.754	0.708	50
	F1	0.662		
Cal D	G1	1.128	1.140	100
	H1	1.152		
Cal E	A2	1.850	1.805	250
	B2	1.760		
Cal F	C2	2.310	2.355	500
	D2	2.400		
Patient	A3	1.009	1.004	**82.9**
	B3	0.999		

Figure 1



*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with CA 19-9 concentrations above 500U/ml may be diluted (for example 1/10 or higher) with CA19-9 zero calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. CA 19-9 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CA 19-9 value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.

13.0 EXPECTED RANGES OF VALUES

The serum CA 19-9 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum or pericardium).

TABLE I
Expected Values for CA 19-9 AccuBind® ELISA Test System

Healthy and non-pregnant subjects	**≤ 40 U/ml**

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the CA 19-9 AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	20	3.1	0.22	7.1%
Level 2	20	28.0	1.42	5.0%
Level 3	20	161.2	4.21	2.6%

TABLE 3
Between Assay Precision* (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	10	3.7	0.34	9.2%
Level 2	10	25.3	1.81	7.1%
Level 3	10	154.0	5.11	3.4%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CA 19-9 AccuBind® ELISA test system has a sensitivity of 1.0 U/ml. The sensitivity was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The CA 19-9 AccuBind® ELISA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 136. The least square regression equation and the correlation coefficient were computed for the CA 19-9 in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (X)	18.62	x = 1.4577+0.8837(y)	0.955
Reference (Y)	19.43		

14.4 Specificity

In order to test the specificity of the antibody pair used massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. No cross reaction was found. Percent cross-reactions for some of these additions are listed below in Table 5.

TABLE 5

Analyte	Concentration	Percent (%) Cross Reaction
CA 19-9	-	100
CA 125	10000 U/ml	0.001
CA 15-3	1000 U/ml	ND*
PSA	5000 ng/ml	ND*
AFP	10000 ng/ml	ND*
CEA	10000 ng/ml	ND*
HCG	10000 mIU/ml	ND*
RF	1000 kIU/ml	ND*

15.0 REFERENCES

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Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP3925 Product Code: *3925-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 (13ml)	2 (13ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (7ml)	2 (7ml)
	H)	1 (8ml)	2 (8ml)

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Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Carcinoembryonic Antigen Next Generation (CEA-Next Generation) Test System

Product Code: 4625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Carcinoembryonic Antigen (CEA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Carcinoembryonic antigen (CEA) is a glycoprotein with a molecular weight of 180 kDA. CEA is the first of the so-called carcinoembryonic proteins that was discovered in 1965 by Gold and Freeman.[1] CEA is the most widely used marker for gastrointestinal cancer.

Although CEA is primarily associated with colorectal cancers, other malignancies that can cause elevated levels of CEA include breast, lung, stomach, pancreas, ovary and other organs. Benign conditions that cause significantly higher than normal levels include inflammation of lung and gastrointestinal (GI) tract and benign liver cancer.[2,3] Heavy smokers, as a group, have higher than normal baseline concentration of CEA.

In this method, CEA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of CEA) are added and the reactants mixed. Reaction between the various CEA antibodies and native CEA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-CEA antibody bound conjugate is separated from the unbound enzyme-CEA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known carcinoembryonic antigen (CEA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with CEA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CEA antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{CEA} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{CEA} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{CEA} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{CEA} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab - Ag_{CEA} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. CEA Next Generation Calibrators – 1ml/vial Icons A-F
Six (6) vials of references CEA Antigen at levels of 0(**A**), 5(**B**), 10(**C**), 25(**D**), 100(**E**) and 250(**F**) ng/ml. A preservative has been added. Store at 2-8°C.
Note: The standards, human serum based, were calibrated using a reference preparation, which was assayed against the 1st International Reference Preparation (IRP# 73/601).

B. CEA Next Generation Enzyme Reagent -13ml/vial -Icon
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, red dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial - Icon S^A
One (1) vial contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

F. Substrate B – 7ml/vial - Icon S^B
One (1) vial contains hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See "Reagent Preparation."

G. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8ºC. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025 & 0.050ml (25µl & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one (1) year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 -27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the CEA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Carcinoembryonic antigen in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CEA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of CEA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 0.320 ng/ml intersects the dose response curve at 18.1 ng/ml CEA concentration (see Figure 1).

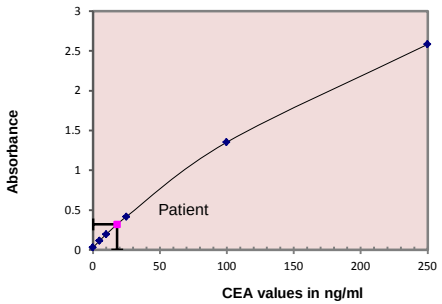
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.028	0.027	0
	B1	0.026		
Cal B	C1	0.115	0.115	5
	D1	0.114		
Cal C	E1	0.196	0.196	10
	F1	0.196		
Cal D	G1	0.432	0.418	25
	H1	0.404		
Cal E	A2	1.403	1.353	100
	B2	1.303		
Cal F	C2	2.580	2.558	250
	D2	2.535		
Patient	E2	0.302	0.320	**18.1**
	F2	0.337		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with CEA concentrations above 250 ng/ml may be diluted (for example 1/10 or higher) with normal male serum (CEA < 5 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. CEA has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CEA value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters. There are patients with colorectal cancer that do not exhibit elevated CEA values and elevated CEA values do not always change with progression or regression of disease. Smokers demonstrate a higher range of baseline values than non-smokers.

13.0 EXPECTED RANGES OF VALUES

Nearly 99% of non-smokers have CEA concentrations less than 5ng/ml. Similarly 99% of smokers have concentrations less than 10ng/ml.[4]

TABLE I
Expected Values for the CEA Next Generation AccuBind® ELISA Test System

Non-smokers	<5ng/ml
Smokers	<10ng/ml

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the CEA Next Generation AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	2.6	0.25	9.6%
Level 2	20	12.5	1.01	8.1%
Level 3	20	24.1	1.35	5.6%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	10	2.8	0.30	10.7%
Level 2	10	12.8	1.18	9.2%
Level 3	10	23.5	1.85	7.8%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CEA Next Generation AccuBind® ELISA test system has a sensitivity of 0.025 ng. This is equivalent to a sample containing 1 ng/ml CEA concentration. The sensitivity was ascertained by determining the variability of the '0 ng/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The CEA Next Generation AccuBind® ELISA method was compared with a reference method. Biological specimens from normal and elevated concentrations were assayed. The total number of such specimens was 64. The values ranged from 0.4 – 128ng/ml. The least square regression equation and the correlation coefficient were computed for the CEA Next Generation AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (X)	10.01	y = 1.17+0.977x	0.995
Reference (Y)	9.04		

E. Specificity:

Highly specific antibodies to CEA molecules have been used in the CEA Next Generation AccuBind® ELISA test system. No interference was detected with the performance of CEA Next Generation AccuBind® ELISA upon addition of massive amounts of the following substances to a human serum pool.

Substance	Concentration
Acetylsalicylic Acid	100 µg/ml
Ascorbic Acid	100 µg/ml
Caffeine	100 µg/ml
AFP	10 µg/ml
PSA	1.0 µg/ml
CA-125	10,000 U/ml
hCG	1000 IU/ml
hLH	10 IU/ml
hTSH	100 mIU/ml
hPRL	100 µg/ml

14.5 Linearity & Hook Effect:

Three different lot preparations of the CEA Next Generation AccuBind® ELISA reagents were used to assess the linearity and hook effect. Massive concentrations of CEA (> 60,000 ng/ml) were used for linear dilutions in pooled human patient sera.

The test showed no hook effect up to concentrations of 60,000 ng/ml and a within dose recovery of 92.0 to 111.4%.

15.0 REFERENCES

1. Gold P, Freedman SO, *J Exp Med*, 121, 439 (1965).
2. Zamcheck N, *Adv Intern Med,* 19, 413 (1974).
3. Rayncao G, Chu TM, *JAMA*, 220, 381 (1972).
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Revision: 3 **Date: 2019-Jul-16** **DCO: 1353**
MP4625 **Product Code: *4625-300***

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
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	G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
3951 DBMaarn, The Neatherlands
www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2°-8° Temperature Limitation Storage Condition (2-8°C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE European Conformity	

MULTI LIGAND CONTROL-TRI LEVEL **PRODUCT CODE: ML-300B**

LOT# MLAC1D3 **EXP: 2026-04-18**

INTENDED USE

The Multi-ligand Controls are intended for use as an assayed quality control material to monitor the consistency of performance of laboratory test procedures associated with determination and monitoring of the clinical status. This product is a human-serum based, lyophilized control, stabilized with preservatives and can be used with all ELISA and CLIA methods.

SUMMARY AND EXPLANATION

The use of quality control material to assist in the assessment of precision in the clinical laboratory is an integral part of laboratory practices. Controls that contain varied levels of analytes are necessary to insure precision and accuracy in immunoassay systems.

REAGENTS

Monobind's Multi-ligand Controls are intended to be used in the exact manner as patient samples. The control is packaged as 6 vials of 3.0 ml, dried. The analyte activities are adjusted to concentrations in the low, middle and high range in order to monitor the efficacy of the procedure in use.

INSTRUCTIONS FOR USE

1) Bring the vials to room temperature before use.
2) Carefully unscrew and remove cap.
3) Add three (3) ml of distilled or deionized water to each vial. Close the cap tightly and let the contents mix thoroughly for 30 minutes
4) Aliquot the materials in 0.5 ml aliquots in cryo vials and store at -20ºC.

STORAGE, STABILITY AND DISPOSAL

This product will be stable until the expiration date when stored unopened at 2 to 8°C. Once the control is reconstituted, all analytes will be stable for 7 days when stored tightly capped at 2 to 8°C with the following exceptions: 1) **C-Peptide, f-PSA, and PRL** should be assayed immediately after reconstitution, and 2) **Folate, Insulin, and PRL-Seq** will be stable for 1 day. To avoid contamination, it is recommended labs aliquot required quantities into vials before each use.

After reconstituting, controls should be tightly capped and returned to refrigerator 2 to 8° C as soon as practical after usage. (Long term room temperature storage is not supported.) After reconstituting, controls should be tightly capped and frozen within 2-hours. **Once thawed, do not refreeze the control; discard remaining materia**l. It is recommended that customers aliquot control into separate containers before freezing to allow for usage on different days. Outdated material should be discarded as a biohazardous component.

STORAGE	STABILITY	TEMPERATURE
Lyophilized, Unopened	Three (3) years	< 8°C
Reconstituted, Opened	Seven (7) days	2 - 8°C
Reconstituted, Opened	Ninety (90) days	< -10°C

EXPECTED RANGE OF VALUES

The mean values printed in this insert were derived from replicate analyses and are specific for this lot of product. The tests listed were performed by Monobind QA using representative lots of this product, as well as those of Monobind's AccuBind® ELISA and AccuLite® CLIA reagents.

Individual laboratory means should fall within the corresponding acceptable range; however laboratory means may vary from the listed values during the life of this control. Therefore, each laboratory should establish its own means and acceptable ranges for the product used, using Monobind's assignment only as guide. A trend log should be maintained for batch to batch consistency of the test. Variations over time and between laboratories may be caused by a) differences in laboratory personnel, b) improper technique, c) instrumentation and reagents, d) improper dilutions from the stated manufacturer's procedure, and/ or e) modifications in the manufacturer's test procedure.

Refer to http://www.monobind.com/site/qc-documents.html for any updated insert information.

WARNING AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE

All products that contain human serum have been found to be non-reactive for HIV 1&2, HIV-Ag, HBsAg, HCV and RPR by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Revision: 1 **Date: 2023-06-15** **Product Code: ML-300B**

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IVD 2°-8° CE 0050

EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Netherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols (EN 980/ISO 15223)

- IVD — In Vitro - Diagnostic Medical Device
- 2°-8° — Temperature Limitation Storage Condition (2-8° C)
- Consult Instructions for Use
- REF — Catalogue Number
- Used By (Expiration Day)
- LOT — Batch Code
- Date of Manufacturer
- Manufacturer
- EC REP — Authorized Rep in European Country
- CE 0050 — European Conformity NB Id #

DOCUMENT HISTORY

PREPARED BY: _____ DEPT: QC VERIFIED BY: AShatoka DEPT:QA

APPROVED BY: _____ DEPT: Admin EFFECTIVE DATE: 2023-06-15

REVISION: 1 DCO: 1624

EXPECTED RANGE OF VALUES FOR MULTI-LIGAND CONTROL - TRI LEVEL

MASTER LOT: MLAC1D3

Analyte	A Range	B Range	C Range	Method
Allergy				
IgE in IU/ml	99.49 ± 32.83	23.24 ± 7.67	158.73 ± 52.38	MB ACCUBIND ELISA
	93.38 ± 30.81	20.30 ± 6.70	159.85 ± 52.75	MB ACCULITE CLIA
Anemia				
Ferritin in ng/ml	27.79 ± 9.17	81.75 ± 26.98	333.07 ± 109.91	MB ACCUBIND ELISA
	24.94 ± 8.23	82.13 ± 27.10	364.67 ± 120.34	MB ACCULITE CLIA
Folate in ng/ml	1.85 ± 0.61	7.90 ± 2.61	12.84 ± 4.24	MB ACCUBIND ELISA
	2.45 ± 0.81	8.58 ± 2.83	13.53 ± 4.46	MB ACCULITE CLIA
Vitamin B12 in pg/ml	286.89 ± 94.67	414.64 ± 136.83	1032.44 ± 340.70	MB ACCUBIND ELISA
	330.71 ± 109.13	428.07 ± 141.26	1021.96 ± 337.25	MB ACCULITE CLIA
Anemia VAST				
(Folate) in ng/ml	2.71 ± 0.89	7.83 ± 2.58	12.49 ± 4.12	MB ACCUBIND ELISA
	2.78 ± 0.92	7.76 ± 2.56	11.62 ± 3.84	MB ACCULITE CLIA
(Vitamin B12) in pg/ml	366.57 ± 120.97	466.13 ± 153.82	978.22 ± 322.81	MB ACCUBIND ELISA
	346.20 ± 114.25	469.55 ± 154.95	919.75 ± 303.52	MB ACCULITE CLIA
Bone Metabolism				
Vit D Direct in ng/ml	28.57 ± 9.43	46.66 ± 15.40	88.77 ± 29.29	MB ACCUBIND ELISA
	31.25 ± 10.31	47.51 ± 15.68	141.73 ± 46.77	MB ACCULITE CLIA
Cancer Markers				
AFP in ng/ml	20.70 ± 6.83	86.38 ± 28.51	190.16 ± 62.75	MB ACCUBIND ELISA
	20.22 ± 6.67	91.03 ± 30.04	195.92 ± 64.65	MB ACCULITE CLIA
CEA in ng/ml	4.02 ± 1.33	19.12 ± 6.31	45.45 ± 15	MB ACCUBIND ELISA
	3.98 ± 1.31	18.12 ± 5.98	48.52 ± 16.01	MB ACCULITE CLIA
CEA Next Generation in ng/ml	4.28 ± 1.41	24.29 ± 8.02	73.41 ± 24.23	MB ACCUBIND ELISA
	3.85 ± 1.27	22.94 ± 7.57	66.64 ± 21.99	MB ACCULITE CLIA
fPSA in ng/ml	0.77 ± 0.25	3.03 ± 1	> 10	MB ACCUBIND ELISA
	0.79 ± 0.26	3.40 ± 1.12	> 10	MB ACCULITE CLIA
tPSA-XS in ng/ml	1.10 ± 0.36	3.63 ± 1.20	23.03 ± 7.60	MB ACCUBIND ELISA
	1 ± 0.33	3.59 ± 1.18	22.99 ± 7.59	MB ACCULITE CLIA
tPSA in ng/ml	1.35 ± 0.44	4.38 ± 1.44	25.63 ± 8.46	MB ACCUBIND ELISA
	1.13 ± 0.37	4.07 ± 1.34	24.77 ± 8.18	MB ACCULITE CLIA
Cancer Markers VAST				
(CEA) in ng/ml	3.70 ± 1.22	18.45 ± 6.09	45.61 ± 15.05	MB ACCUBIND ELISA
	3.33 ± 1.10	16.74 ± 5.53	46.28 ± 15.27	MB ACCULITE CLIA
(AFP) in ng/ml	20.81 ± 6.87	92.04 ± 30.37	189.19 ± 62.43	MB ACCUBIND ELISA
	19.70 ± 6.50	82.27 ± 27.15	184.37 ± 60.84	MB ACCULITE CLIA
(tPSA) in ng/ml	1.23 ± 0.40	4.29 ± 1.42	30.77 ± 10.16	MB ACCUBIND ELISA
	1.08 ± 0.36	4.24 ± 1.40	29.32 ± 9.68	MB ACCULITE CLIA
Cardiac Markers				
Dig in ng/ml	0.36 ± 0.12	1.69 ± 0.56	2.68 ± 0.88	MB ACCUBIND ELISA
	0.46 ± 0.15	1.61 ± 0.53	2.84 ± 0.94	MB ACCULITE CLIA
Diabetes				
C-Peptide in ng/ml	0.48 ± 0.16	2.38 ± 0.79	4.56 ± 1.50	MB ACCUBIND ELISA
	0.44 ± 0.15	2.29 ± 0.75	4.19 ± 1.38	MB ACCULITE CLIA
Insulin in µIU/ml	28.97 ± 9.56	82.75 ± 27.31	169.78 ± 56.03	MB ACCUBIND ELISA
	26.90 ± 8.88	84.63 ± 27.93	162.90 ± 53.76	MB ACCULITE CLIA
Rapid Insulin in µIU/ml	28.02 ± 9.25	81.63 ± 26.94	159.35 ± 52.59	MB ACCUBIND ELISA
Fertility				
FSH in mIU/ml	8.64 ± 2.85	24.11 ± 7.96	42.71 ± 14.09	MB ACCUBIND ELISA
	7.92 ± 2.61	23.58 ± 7.78	41.52 ± 13.70	MB ACCULITE CLIA
hCG in mIU/ml	4.43 ± 1.46	24.10 ± 7.95	146.28 ± 48.27	MB ACCUBIND ELISA
	4.35 ± 1.74	22.95 ± 7.57	151.35 ± 49.95	MB ACCULITE CLIA
hCG-XR in mIU/ml	4.18 ± 1.38	28.67 ± 9.46	143.88 ± 47.48	MB ACCUBIND ELISA
	3.56 ± 1.17	28.58 ± 9.43	155.85 ± 51.43	MB ACCULITE CLIA
LH in mIU/ml	3.88 ± 1.28	22.25 ± 7.34	53.53 ± 17.67	MB ACCUBIND ELISA
	3.38 ± 1.12	20.07 ± 6.62	53.08 ± 21.72	MB ACCULITE CLIA
PRL in ng/ml	5.08 ± 1.68	24.36 ± 8.04	38.50 ± 12.70	MB ACCUBIND ELISA
	4.78 ± 1.58	21.14 ± 6.98	36.80 ± 12.14	MB ACCULITE CLIA
PRL-seq in ng/ml	4.24 ± 1.41	20.16 ± 6.65	35.96 ± 11.87	MB ACCUBIND ELISA
	4.14 ± 1.37	18.63 ± 6.15	36.61 ± 12.08	MB ACCULITE CLIA
Rapid HCG in mIU/ml	4.70 ± 1.55	27.56 ± 9.10	188.52 ± 62.21	MB ACCUBIND ELISA
Fertility VAST				
(FSH) in mIU/ml	6.84 ± 2.26	18.59 ± 6.13	32.98 ± 10.88	MB ACCUBIND ELISA
	6.12 ± 2.02	17.63 ± 5.82	37 ± 12.21	MB ACCULITE CLIA
(LH) in mIU/ml	4.29 ± 1.42	22.36 ± 7.38	50.17 ± 16.56	MB ACCUBIND ELISA
	3.74 ± 1.23	20.37 ± 6.72	43.9 ± 14.49	MB ACCULITE CLIA
(hCG) in mIU/ml	4.88 ± 1.61	24.08 ± 7.95	144.63 ± 47.73	MB ACCUBIND ELISA
	5.92 ± 1.95	26.53 ± 8.76	149.81 ± 49.44	MB ACCULITE CLIA
Triple Screen VAST				
(AFP) in ng/ml	21.47 ± 7.09	103.20 ± 34.05	188.63 ± 62.25	MB ACCUBIND ELISA
	19.13 ± 6.31	100.30 ± 33.10	203.38 ± 67.11	MB ACCULITE CLIA
(uE3) in ng/ml	1.11 ± 0.37	3.32 ± 1.10	5.99 ± 1.98	MB ACCUBIND ELISA
	1.10 ± 0.36	2.72 ± 0.90	5.40 ± 1.78	MB ACCULITE CLIA
(hCG) in mIU/ml	4.29 ± 1.41	23.43 ± 7.73	149.04 ± 49.18	MB ACCUBIND ELISA
	4.78 ± 1.58	21.30 ± 7.03	174.85 ± 57.70	MB ACCULITE CLIA
Growth Deficiency				
hGH in µIU//ml	5.29 ± 1.75	32.33 ± 10.67	67.95 ± 22.42	MB ACCUBIND ELISA
	5.01 ± 1.65	32.03 ± 10.57	68 ± 23	MB ACCULITE CLIA
Steroids				
Aldosterone in ng/ml	51.72 ± 17.43	471.16 ± 155.48	1195.18 ± 394.41	MB ACCUBIND ELISA
	60.35 ± 19.92	447.70 ± 147.74	1167.75 ± 385.36	MB ACCULITE CLIA
ANST in ng/ml	1 ± 0.33	1.52 ± 0.50	10.60 ± 3.50	MB ACCUBIND ELISA
	0.89 ± 0.29	1.36 ± 0.45	11.45 ± 3.78	MB ACCULITE CLIA
Cortisol in µg/dl	2.43 ± 0.80	13.98 ± 4.61	30.98 ± 11.40	MB ACCUBIND ELISA
	3.02 ± 1	14.91 ± 4.92	33.37 ± 11.01	MB ACCULITE CLIA
DHEA-S in µg/ml	0.37 ± 0.12	1.64 ± 0.54	4.40 ± 1.45	MB ACCUBIND ELISA
	0.40 ± 0.17	1.51 ± 0.50	3.99 ± 1.32	MB ACCULITE CLIA
DHEA in ng/ml	0.89 ± 0.30	2.94 ± 0.97	12.42 ± 4.10	MB ACCUBIND ELISA
	1.02 ± 0.34	3.34 ± 1.10	14.14 ± 4.67	MB ACCULITE CLIA
E1 in ng/ml	32 ± 13.02	149.61 ± 49.37	365.28 ± 120.54	MB ACCUBIND ELISA
E2 in pg/ml	36.26 ± 11.96	180.72 ± 59.64	295.83 ± 97.62	MB ACCUBIND ELISA
	35.85 ± 11.83	189 ± 62.37	283.05 ± 93.41	MB ACCULITE CLIA
uE3 in ng/ml	1.04 ± 0.41	2.43 ± 0.80	5.14 ± 1.70	MB ACCUBIND ELISA
	1.19 ± 0.39	2.51 ± 0.83	4.97 ± 1.64	MB ACCULITE CLIA
Progesterone in ng/ml	0.97 ± 0.33	7.20 ± 2.37	25.05 ± 8.27	MB ACCUBIND ELISA
	1.01 ± 0.33	7.10 ± 2.34	25.39 ± 8.38	MB ACCULITE CLIA
17-OHP in ng/ml	0.62 ± 0.20	2.01 ± 0.66	5.67 ± 1.87	MB ACCUBIND ELISA
	0.71 ± 0.24	2.07 ± 0.68	5.71 ± 1.89	MB ACCULITE CLIA
17-OHP-SI in ng/ml	0.36 ± 0.12	1.13 ± 0.37	3 ± 0.99	MB ACCUBIND ELISA
	0.4 ± 0.13	1 ± 0.33	2.90 ± 0.96	MB ACCULITE CLIA
Testosterone in ng/ml	0.28 ± 0.09	1.03 ± 0.34	6.93 ± 2.29	MB ACCUBIND ELISA
	0.42 ± 0.14	0.90 ± 0.30	7.93 ± 2.62	MB ACCULITE CLIA
Free Testosterone (0-60pg/ml calibration)	1.11 ± 0.37	3.46 ± 1.14	28.89 ± 9.53	MB ACCUBIND ELISA
	1.21 ± 0.40	3.69 ± 1.22	31.29 ± 10.32	MB ACCULITE CLIA
Thyroid				
T3 in ng/ml	0.51 ± 0.17	1.15 ± 0.38	3.27 ± 1.08	MB ACCUBIND ELISA
	0.52 ± 0.17	1.17 ± 0.39	3.17 ± 1.05	MB ACCULITE CLIA
T4 in µg/dl	2.90 ± 0.96	7.53 ± 2.48	16.91 ± 5.58	MB ACCUBIND ELISA
	2.90 ± 0.96	8.37 ± 2.76	16.42 ± 5.42	MB ACCULITE CLIA
TSH in µIU/ml	0.97 ± 0.32	6.50 ± 2.14	34.20 ± 11.29	MB ACCUBIND ELISA
	0.88 ± 0.29	6.15 ± 2.03	31.97 ± 10.55	MB ACCULITE CLIA
fT3 in pg/ml	1.58 ± 0.52	3.46 ± 1.14	6.58 ± 2.17	MB ACCUBIND ELISA
	1.62 ± 0.78	3.52 ± 1.16	6.78 ± 2.24	MB ACCULITE CLIA
fT4 in ng/dl	0.36 ± 0.12	1.76 ± 0.58	3.73 ± 1.23	MB ACCUBIND ELISA
	0.38 ± 0.12	1.63 ± 0.54	3.23 ± 1.07	MB ACCULITE CLIA
T3-Uptake in %U	25.49 ± 2.81	33.22 ± 2.94	46.13 ± 2.95	MB ACCUBIND ELISA
	26.63 ± 2.37	34.60 ± 2.43	49 ± 6.93	MB ACCULITE CLIA
Rapid TSH in µIU/ml	0.87 ± 0.29	6.45 ± 2.13	34.26 ± 11.31	MB ACCUBIND ELISA
	0.77 ± 0.25	6.18 ± 2.04	31.20 ± 10.30	MB ACCULITE CLIA
Thyroid VAST				
(TSH) in µIU/ml	0.98 ± 0.32	7.08 ± 2.34	38.39 ± 12.67	MB ACCUBIND ELISA
	0.94 ± 0.34	6.98 ± 2.30	35.98 ± 11.87	MB ACCULITE CLIA
Strep T3 in ng/ml	0.56 ± 0.18	1.29 ± 0.43	2.88 ± 0.95	MB ACCUBIND ELISA
	0.63 ± 0.22	1.24 ± 0.48	2.65 ± 0.88	MB ACCULITE CLIA
Strep T4 in µg/dl	2.90 ± 0.96	9.11 ± 3.01	13.56 ± 4.47	MB ACCUBIND ELISA
	3.08 ± 1.02	9.32 ± 3.08	12.51 ± 4.13	MB ACCULITE CLIA
Free Thyroid VAST				
(TSH) in µIU/ml	0.79 ± 0.26	7.37 ± 2.43	33.82 ± 11.16	MB ACCUBIND ELISA
	0.78 ± 0.26	7.23 ± 2.39	33.38 ± 11.01	MB ACCULITE CLIA
Strept fT3 in pg/ml	1.43 ± 0.47	3.86 ± 1.27	8.24 ± 2.72	MB ACCUBIND ELISA
	1.59 ± 0.52	4.12 ± 1.36	8.26 ± 2.73	MB ACCULITE CLIA
Strept fT4 in ng/dl	0.31 ± 0.10	1.63 ± 0.54	2.82 ± 0.93	MB ACCUBIND ELISA
	0.33 ± 0.17	1.35 ± 0.45	3.03 ± 1	MB ACCULITE CLIA

THYROGLOBULIN (TG) CONTROL - TRI LEVEL **PRODUCT CODE: TG-300**
LOT# TGAC1H3 **EXP: 2026-08-01**

INTENDED USE
The Thyroglobulin Controls are intended for use as an assayed quality control material to monitor the consistency of performance of laboratory test procedures associated with determination and monitoring of thyroglobulin levels. This product is human-serum based, liquid control, stabilized with preservatives and can be used with all RIA, EIA, ELISA, CLIA, and FIA methods.

SUMMARY AND EXPLANATION
The use of quality control material to assist in the assessment of precision in the clinical laboratory is an integral part of laboratory practices. Controls that contain varied levels of analytes are necessary to insure precision and accuracy in immunoassay systems.

REAGENTS
Monobind's thyroglobulin controls are intended to be used in the exact manner as patient samples. The control is packaged as 6 vials of 3.0 ml (2 of each level). The analyte activities are adjusted to concentrations in the low, middle and high range in order to monitor the efficacy of the procedure in use.

INSTRUCTIONS FOR USE
1) Bring the vials to room temperature before use.
2) Carefully unscrew and remove cap.
3) Aliquot the materials in 0.5 ml aliquots in cryo vials and store at ≤ -20ºC.

STORAGE, STABILITY AND DISPOSAL
The control is provided liquid and ready to use. This product will be stable until the expiration date when stored unopened at **< - 20.** Once the control is opened, all analytes will be stable for 30 days when stored tightly capped at 2-8 °C. To avoid contamination, it is recommended labs aliquot required quantities into vials before each use.

Long term room temperature storage is not supported. Unused controls should be tightly capped and frozen within two (2) hours. Once thawed, do not refreeze the control; discard remaining material. It is recommended that customers aliquot control into separate containers before freezing to allow for usage on different days. Outdated material should be discarded as a biohazardous component.

STORAGE	STABILITY	TEMPERATURE
Unopened	Three (3) years	≤ -20ºC
Opened	Thirty (30) days	2-8°C

ASSIGNMENT OF VALUES & EXPECTED RANGE OF VALUES

EXPECTED RANGE OF VALUES FOR Thyroglobulin Controls - Tri-level Set MASTER LOT TGAC1H3				
Analyte	A Range	B Range	C Range	Method
Tg in ng/ml	2.44 ± 0.80	11.60 ± 3.83	57.59 ± 19.0	MB ACCUBIND ELISA
	1.78 ± 0.59	10.03 ± 3.31	52.75 ± 17.41	MB ACCULITE CLIA

The mean values printed in this insert were derived from replicate analyses and are specific for this lot of product. The tests listed were performed by Monobind QC using representative lots of this product, as well as those of Monobind's AccuBind® ELISA and AccuLite® CLIA reagents.

Individual laboratory means should fall within the corresponding acceptable range; however, laboratory means may vary from the listed values during the life of this control. Therefore, each laboratory should establish its own means and acceptable ranges for the product used, using Monobind's assignment only as guide. A trend log should be maintained for batch to batch consistency of the test. Variations over time and between laboratories may be caused by a) differences in laboratory personnel, b) improper technique, c) instrumentation and reagents, d) improper dilutions from the manufacturer's stated procedure, and/ or e) modifications in the manufacturer's test procedure.

Refer to http://www.monobind.com/site/qc-documents.html for any updated insert information.

WARNING AND PRECAUTIONS
FOR IN VITRO DIAGNOSTIC USE
All products that contain human serum have been found to be negative and non reactive for HIV 1&2, HIV-Ag, HBsAg, HCV and RPR by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Revision: 0 **Date: 2023-08-01** **Product Code: TG-300**

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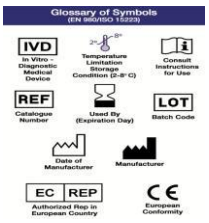

DOCUMENT HISTORY
PREPARED BY: _____ DEPT: QC VERIFIED BY: _____ DEPT:QA
APPROVED BY: _____ DEPT: Admin EFFECTIVE DATE: 2023-08-01
REVISION: 0 DCO: N/A

Tumor Marker Control **PRODUCT CODE: TMC-300**

LOT# TMCAC1K1 **EXP: 2024/11/30**

INTENDED USE

The Tumor Marker Controls are intended for use as an assayed quality control material to monitor the consistency of performance of laboratory test procedures associated with determination and monitoring of the clinical status. This product is a human-serum based, liquid control, stabilized with preservatives and can be used with all ELISA and CLIA methods.

SUMMARY AND EXPLANATION

The use of quality control material to assist in the assessment of precision in the clinical laboratory is an integral part of laboratory practices. Controls that contain varied levels of analytes are necessary to insure precision and accuracy in immunoassay systems.

REAGENTS

Monobind's The Tumor Marker Controls are intended to be used in the exact manner as patient samples. The control is packaged as 6 vials of 2.0 ml. The analyte activities are adjusted to concentrations in the low, middle and high range in order to monitor the efficacy of the procedure in use.

INSTRUCTIONS FOR USE

1) Bring the vials to room temperature before use.
2) Carefully unscrew and remove cap.
3) Aliquot the materials in 0.5 ml aliquots in cryo vials and store at -20ºC.

STORAGE, STABILITY AND DISPOSAL

This control is provided liquid and ready to use. This product will be stable until the expiration date when stored unopened at <-20°C. Once the control is opened, all analytes will be stable for 7 days when stored tightly capped at 2 to 8°C. To avoid contamination, it is recommended labs aliquot required quantities into vials before each use.

Controls should be tightly capped and returned to refrigerator 2 to 8° C as soon as practical after usage. (Long term room temperature storage is not supported.) Unused controls should be tightly capped and frozen within two (2) hours. Once thawed, do not refreeze the control; discard remaining material. It is recommended that customers aliquot control into separate containers before freezing to allow for usage on different days. Outdated material should be discarded as a biohazardous component.

STORAGE	STABILITY	TEMPERATURE
Unopened	Three (3) years	< -20°C
Unopened	Ninety (90) days	2 – 8°C
Opened	Seven (7) days	2 – 8°C

EXPECTED RANGE OF VALUES

The mean values printed in this insert were derived from replicate analyses and are specific for this lot of product. The tests listed were performed by Monobind QA using representative lots of this product, as well as those of Monobind's AccuBind® ELISA and AccuLite® CLIA reagents.

Analyte	A Range	B Range	C Range	Method
CA 125 in U/ml	15.69 ± 5.18	60.31 ± 19.90	117.81 ± 38.88	MB ACCUBIND ELISA
	18.16 ± 5.99	64.25 ± 21.20	126.93 ± 41.89	MB ACCULITE CLIA
CA 19-9 in U/ml	14.89 ± 5.43	52.19 ± 17.22	91.50 ± 30.19	MB ACCUBIND ELISA
	15.01 ± 5.08	46.89 ± 15.47	80.73 ± 26.64	MB ACCULITE CLIA
CA 15-3 in U/ml	15.50 ± 5.12	47.47 ± 15.67	93.09 ± 30.72	MB ACCUBIND ELISA
	14.63 ± 4.83	44.10 ± 19.09	105.29 ± 34.75	MB ACCULITE CLIA

Individual laboratory means should fall within the corresponding acceptable range; however laboratory means may vary from the listed values during the life of this control. Therefore, each laboratory should establish its own means and acceptable ranges for the product used, using Monobind's assignment only as guide. A trend log should be maintained for batch to batch consistency of the test. Variations over time and between laboratories may be caused by a) differences in laboratory personnel, b) improper technique, c) instrumentation and reagents, d) improper dilutions from the stated manufacturer's procedure, and/ or e) modifications in the manufacturer's test procedure.

Refer to http://www.monobind.com/site/qc-documents.html for any updated insert information.

WARNING AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE

All products that contain human serum have been found to be non-reactive for HIV 1&2, HIV-Ag, HBsAg, HCV and RPR by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Revision: 0 **Date: 2021-NOV-30** **Product Code: TMC-300**

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Please visit our website to learn more about our other interesting products and services.

IVD

CEpartner4U, Esdoornlaan 13, 3951DB Maarn, The Netherlands www.cepartner4u.eu

EC REP

Glossary of Symbols (EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device

Temperature Limitation Storage Condition (2-8° C)

Consult Instructions for Use

REF — Catalogue Number

Date of Manufacturer

LOT — Batch Code

Used By (Expiration Day)

Manufacturer

European Conformity

EC REP — Authorized Rep in European Country

DOCUMENT HISTORY

PREPARED BY: _____ DEPT: QC VERIFIED BY: _____ DEPT: QA

APPROVED BY: _____ DEPT: Administration EFFECTIVE DATE: 2021-NOV-30

REVISION: 0 DCO: N/A

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Free Prostate Specific Antigen (fPSA) Test System

Product Code: 2325-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Free Prostate Specific Antigen (fPSA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prostate Specific antigen (PSA) is a serine protease with chymotrypsin-like activity.[1,2] The protein is a single chain glycoprotein with a molecular weight of 28.4 kDA.[3] PSA derives its name from the observation that it is a normal antigen of the prostate, but is not found in any other normal or malignant tissue. PSA is released from the normal prostate and appears at low serum concentrations in healthy men. Studies with reverse transcription-PCR have shown that PSA also is expressed at a low concentration in peripheral blood cells and other tissues.[4] High serum concentrations can be detected in patients with advanced prostate cancer (PCA).[5] Therefore, PSA is applied as a tumor marker for the clinical management of PCA.[6] However, increased PSA concentrations in serum also occur in patients with benign prostate hyperplasia (BPH).[7] Hence the goal is to discriminate clearly between BPH and PCA in the clinical laboratory to spare the patient invasive diagnostic procedures, such as a prostate biopsy.

In human serum, PSA occurs in two forms: free PSA (fPSA) and complexed PSA. The major form is a complex of PSA and α_1-antichymotrypsin (ACT). The fraction of fPSA was shown to be substantially smaller in patients with untreated PCA than in patients with BPH. Therefore, combined measurements of fPSA and total PSA (tPSA) may lead to a better discrimination between BPH and PCA Some recent studies have already shown that the fPSA/tPSA ratio is helpful in the differential diagnosis of BPH and PCA.

PSA is found in benign, malignant and metastatic prostate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found to be more useful than prostatic acid phosphatase (PAP) in the diagnosis and management of patients due to increased sensitivity.[4]

In this method, fPSA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different free epitopes of fPSA) are added and the reactants mixed. Reaction between the various fPSA antibodies and native fPSA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-fPSA antibody bound conjugate is separated from the unbound enzyme-fPSA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known prostate specific antigen (fPSA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with fPSA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PSA antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(P)} + Ag_{fPSA} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{fPSA} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Antibody (Excess Quantity)
Ag_{fPSA} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)} - Ag_{fPSA} - {}^{Btn}Ab_{(m)}$ =Antigen-Antibodies Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)} - Ag_{fPSA} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. fPSA Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum references free PSA antigen at levels of 0(**A**), 0.5(**B**), 1.0(**C**), 2.5(**D**), 5.0(**E**) and 10.0(**F**) ng/ml. A preservative has been added. Store at 2-8°C.

Note: The calibrators, protein based buffered matrix, were calibrated using a reference preparation, which was assayed against the WHO 1st International Standard 96/668.

B. fPSA Enzyme Reagent – 13 ml/vial - Icon
One (1) vial containing enzyme labeled antibody, biotinylated specific free PSA monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C. (see Reagent Preparation Section).

E. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

Materials Required But Not Provided:
1. Pipette capable of delivering 0.50 & 0.100ml (50 & 100µl) volume with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at room temperature (2-30°C) for up to 60 days.
2. **Working Substrate Solution – Stable for one year**
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the fPSA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature (20-27°C).
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of fPSA in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding fPSA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of fPSA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.648) intersects the dose response curve at 2.28ng/ml fPSA concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such

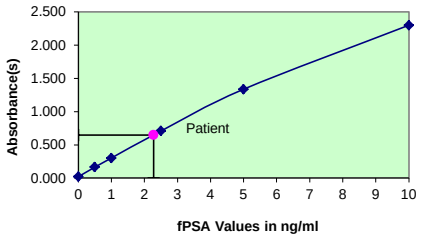
software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.019	0.021	0
	B1	0.022		
Cal B	C1	0.167	0.164	0.5
	D1	0.161		
Cal C	E1	0.300	0.302	1.0
	F1	0.304		
Cal D	G1	0.701	0.707	2.5
	H1	0.714		
Cal E	A2	1.353	1.337	5.0
	B2	1.321		
Cal F	C2	2.286	2.300	10.0
	D2	2.314		
Patient	E2	0.647	0.648	2.28
	F2	0.648		

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with fPSA concentrations above 10 ng/ml may be diluted (for example 1/10 or higher) with normal female serum (PSA = 0 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, is essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis, as required by CE Mark IVD Directive 98/79/EC, for this and other devices made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (*Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33).* For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. fPSA is elevated in benign prostatic hyperplasia (BPH). Clinically an elevated fPSA value alone is not of diagnostic value as a specific test for differential diagnosis of BPH. The ratio of fPSA/tPSA is a better marker and should be used in conjunction with other clinical observations (DRE) and diagnostic procedures (prostate biopsy).
8. When the total PSA (tPSA) reads 4-10 ng/ml, the fPSA/tPSA ratio is useful in the differential diagnosis of BPH and PC (Prostate Cancer). Depending on the ratio, the probability can be determined as follows:

fPSA/tPSA Ratio	Probability of Prostate Cancer
0-10%	55%
10-15%	28%
15- 20%	25%
> 20%	10%

13.0 EXPECTED RANGE OF VALUES

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

TABLE I
Expected Values for the fPSA AccuBind® ELISA Test System

Healthy Males	≤ 1.3 ng/ml

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the fPSA AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	0.48	0.03	5.6%
Level 2	20	1.83	0.10	5.3%
Level 3	20	11.35	0.47	4.2%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	0.53	0.05	9.4%
Level 2	20	1.93	0.14	7.2%
Level 3	20	>11	-	-

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The theoretical sensitivity, or minimum detection limit, calculated by the interpolation of the mean plus two standard deviations of 20 replicates of the 0 ng/ml fPSA calibrator, is 0.008 ng/ml.

14.3 Accuracy

The fPSA AccuBind® ELISA test system was compared with a reference method. Clinical and non-clinical biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 167. The least square regression equation and the correlation coefficient were computed for the fPSA AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (x)	1.62	x = 0.0189 + 0.9649(y)	0.957
Reference (y)	1.66		

Only slight amounts of bias between the fPSA AccuBind® ELISA test system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity:

The following substances did not interfere with the performance of fPSA determination using the fPSA AccuBind® ELISA test system. These substances were added to the pooled sera in concentrations 10-100 times more than normal.

Compound	*Concentration Added*
AFP	10 µg/ml
Atropine	100 µg/ml
Acetylsalicylic Acid	100 µg/ml
Ascorbic Acid	100 µg/ml
Caffeine	100 µg/ml
Dexamethasone	10 µg/ml
Flutamide	100 µg/ml
hCG	100 IU/ml
hLH	100 IU/ml
Methotrexate	100 µg/ml
Prolactin	100 µg/ml
TSH	100 mIU/ml

15.0 REFERENCES

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Effective Date: 2019-Jul-16 Rev 5 **DCO: 1353**
MP2325 **Product Code: 2325-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2° 8° CE 0050

EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8°C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE 0050 — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Total Prostate Specific Antigen (PSA) Test System

Product Code: 2125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Prostate Specific Antigen (PSA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prostate Specific Antigen (PSA) is a serine protease with chymotrypsin-like activity.[1,2] The protein is a single chain glycoprotein with a molecular weight of 28.4 kDa.[3] PSA derives its name from the observation that it is a normal antigen of the prostate, but is not found in any other normal or malignant tissue.

PSA is found in benign, malignant and metastatic prostate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found to be more useful than prostatic acid phosphatase (PAP) in the diagnosis and management of patients due to increased sensitivity.[4]

In this method, PSA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of PSA) are added and the reactants mixed. Reaction between the various PSA antibodies and native PSA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-PSA antibody bound conjugate is separated from the unbound enzyme-PSA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known total prostate specific antigen (PSA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with PSA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PSA antibody.
Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + Ag_{PSA} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ =Biotinylated Antibody (Excess Quantity)
Ag_{PSA} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$ =Antigen-Antibodies Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$+Streptavidin$_{C.W.}$⇒Immobilized complex
Streptavidin$_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. PSA Calibrators – 1 ml/vial – Icons A-F
Six (6) vials of serum references PSA Antigen at levels of 0(**A**), 5(**B**), 10(**C**), 25(**D**), 50(**E**) and 100(**F**) ng/ml. A preservative has been added. Store at 2-8°C.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the 1st IS 96/670.

B. PSA Enzyme Reagent – 13 ml/vial – Icon E
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C. (see Reagent Preparation Section).

E. Substrate A – 7 ml/vial – Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7 ml/vial – Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. (see Reagent Preparation Section).

G. Stop Solution – 8 ml/vial – Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8ºC. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a 96-well microplate. For other kit configurations, refer to the table at the end of this insert.

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025, 0.050 & 0.100 ml (25, 50, & 100 µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate covers for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050 ml (50 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 - 27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the PSA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PSA in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding PSA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of PSA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.142) intersects the dose response curve at (23.6 ng/ml) PSA concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

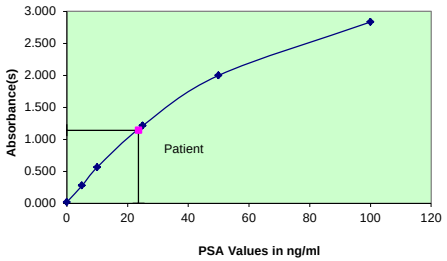
EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.019	0.019	0
	B1	0.019		
Cal B	C1	0.279	0.276	5
	D1	0.273		
Cal C	E1	0.567	0.563	10
	F1	0.559		
Cal D	G1	1.248	1.213	25
	H1	1.179		
Cal E	A2	2.051	1.999	50
	B2	1.947		
Cal F	C2	2.892	2.833	100
	D2	2.775		
Patient	E2	1.186	1.142	23.6

	F2	1.099		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with PSA concentrations above 100 ng/ml may be diluted (for example 1/10 or higher) with normal female serum (PSA = 0 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interactions between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato, LM, Stuart, MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin. Chem.* 1988: 3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. PSA is elevated in benign prostate hypertrophy (BPH). Clinically, an elevated **PSA value alone is not of diagnostic value as a specific test for cance**r and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures (prostate biopsy). Free PSA determinations may be helpful in regard to the discrimination of BPH and prostate cancer conditions.[5]
8. Due to the variation in the calibration used in PSA/ fPSA test kits and differences in epitopic recognition of different antibodies, it is always suggested that the patient sample should be tested with PSA/ fPSA tests made by the same manufacturer**. (*Monobind Inc. offers a fPSA ELISA test that should be used for consistency reasons, when needed.*)**

13.0 PERFORMANCE CHARACTERISTICS

Healthy males are expected to have values below 4 ng/ml.[4]

TABLE I
Expected Values for PSA AccuBind® ELISA Test System

Healthy Males	<4 ng/ml

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal"-persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the PSA AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	1.06	0.06	5.2%
Level 2	20	3.56	0.18	5.1%
Level 3	20	23.07	0.88	3.8%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	0.98	0.08	8.5%
Level 2	20	3.35	0.19	5.7%
Level 3	20	23.17	0.95	4.1%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The PSA AccuBind® ELISA test system has a sensitivity of 0.0003 ng/well. This is equivalent to a sample containing 0.013 ng/ml PSA concentration.

14.3 Accuracy

The PSA AccuBind® ELISA test system was compared with a reference Elisa method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 241. The least square regression equation and the correlation coefficient were computed for the PSA AccuBind® ELISA test method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (X)	5.62	y = -0.0598+0.98(X)	0.987
Reference (Y)	5.57		

Only slight amounts of bias between the PSA AccuBind® ELISA test system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity:

No interference was detected with the performance of PSA AccuBind® ELISA test system upon addition of massive amounts of the following substances to a human serum pool.

Substance	Concentration
Acetylsalicylic Acid	100 µg/ml
Ascorbic Acid	100 µg/ml
Caffeine	100 µg/ml
CEA	10 µg/ml
AFP	10 µg/ml
CA-125	10,000 U/ml
hCG	1000 IU/ml
hLH	10 IU/ml
hTSH	100 mIU/ml
hPRL	100 µg/ml

15.0 REFERENCES

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6. Prestigiacomo AF, Stamey TA, "Physiological variations of serum prostate antigen in the (4-10 ng/ml) range in male volunteers', *J Urol*, 155, 1977-80 (1996).
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Revision: 6 Date: 2022-MAY-01 DCO: 1557
MP2125 Product Code: *2125-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE 0050 — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Thyrotropin (TSH) Test System
Product Code: 325-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyrotropin Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 Daltons and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism.[1,2] The structure of human TSH is similar to that of the pituitary and placental gonadotropins, consisting of an 89-amino acid α-subunit which is similar or identical between these hormones and a 115-amino acid β-subunit, which apparently confers hormonal specificity. The production of the 2 subunits is separately regulated with apparent excess production of the α-subunit. The TSH molecule has a linear structure consisting of the protein core with carbohydrate side chains; the latter accounts for 16% of the molecular weight.

TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduces the response of the pituitary to the stimulatory effects of TRH. In secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH levels are generally low or normal. Either pituitary TSH deficiency (secondary hypothyroidism) or insufficiency of stimulation of the pituitary by TRH (tertiary hypothyroidism) causes this. The TRH stimulation test differentiates these conditions. In secondary hypothyroidism, TSH response to TRH is blunted while a normal or delayed response is obtained in tertiary hypothyroidism.

Further, the advent of immunoenzymometric assays has provided the laboratory with sufficient sensitivity to enable the differentiating of hyperthyroidism from euthyroid population and extending the usefulness of TSH measurements. This method is a second-generation assay, which provides the means for discrimination in the hyperthyroid-euthyroid range. The functional sensitivity (<20% between assay CV) of the one-hour procedure is 0.195 µIU/ml while the two-hour procedure has a functional sensitivity of 0.095µIU/ml.[3]

In this method, TSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies are added and the reactants mixed. Reaction between the various TSH antibodies and native TSH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the antibody bound enzyme-thyrotropin conjugate is separated from the unbound enzyme-thyrotropin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known thyrotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with thyrotropin concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + Ag_{TSH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{TSH} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme -Polyclonal Antibody (Excess Quantity)
$^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobolized on well
Immobilized complex = sandwich complex bound to the well surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. TSH Calibrators – 1ml/vial - Icons A-G
Seven (7) vials of references for TSH Antigen at levels of 0(**A**), 0.5(**B**), 2.5(**C**), 5.0(**D**), 10(**E**), 20(**F**) and 40(**G**) µIU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IRP 80/558.

B. TSH Enzyme Reagent – 13ml/vial - Icon
One (1) vial containing enzyme labeled affinity purified polyclonal goat antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5% (optional).
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Storage container for storage of wash buffer.
10. Distilled or deionized water.
11. Quality Control Materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, (100µl) 0.100 ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal, and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the dose response curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or de-ionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the TSH Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature. ******
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

** For better low-end sensitivity (< 0.5µIU/ml), incubate 120 minutes at room temperature. The 40µIU/ml calibrator should be excluded since absorbance over 3.0 units will be experienced. Follow the remaining steps.

Note: Dilute samples reading over 40 µIU/ml by 1:5 and 1:10 with TSH '0' Calibrator. Multiply the results by the dilution factor to obtain accurate results.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of thyrotropin in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1
2. Plot the absorbance for each duplicate serum reference versus the corresponding TSH concentration in µIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

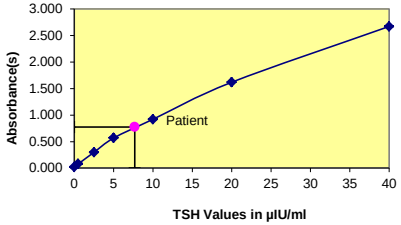
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of TSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.775) intersects the dose response curve at (7.66 µIU/ml) TSH concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs	Mean Abs	Value (µIU/ml)
Cal A	A1	0.018	0.019	0
	B1	0.021		
Cal B	C1	0.076	0.079	0.5
	D1	0.082		
Cal C	E1	0.302	0.298	2.5
	F1	0.293		
Cal D	G1	0.556	0.567	5.0
	H1	0.577		
Cal E	A2	0.926	0.921	10
	B2	0.916		
Cal F	C2	1.610	1.619	20
	D2	1.629		
Cal G	E2	2.694	2.671	40
	F2	2.647		
Control	G2	0.800	0.775	**7.66**
	H2	0.751		
Patient	A3	1. 391	1.383	**16.65**
	B3	1.375		

Figure 1



*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance of calibrator 'G' (40 µIU/ml) should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. Patient specimens with TSH concentrations over 40µIU/ml may be diluted (1:5 or 1:10) with the '0' calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurement and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. Serum TSH concentration is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. **Thus, thyrotropin concentration alone is not sufficient to assess clinical status.**
7. Serum TSH values may be elevated by pharmacological intervention. Domperiodone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.
8. A decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxine.[4]
9. Genetic variations or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due to the reactivity of the antibodies involved.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the TSH AccuBind® ELISA Test System. The number and determined range are given in Table 1. A nonparametric method (95% Percentile Estimate) was used.

TABLE I
Expected Values for the TSH ELISA Test System (in µIU/ml)

Number	139	2.5 Percentile–70% Conf Int	
Low Normal	0.39	Low Range	0.28 – 0.53
High Normal	6.16	High Range	5.60 – 6.82

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the TSH AccuBind® test system were determined by analyses on three different levels of pool control sera. The number (N), mean (X) value, standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in µIU/ml)

Sample	N	X	σ	C.V.
Pool 1	24	0.37	0.03	8.1%
Pool 2	24	6.75	0.43	6.4%
Pool 3	24	29.30	1.94	6.6%

TABLE 3
Between Assay Precision* (Values in µIU/ml)

Sample	N	X	σ	C.V.
Pool 1	10	0.43	0.04	9.3%
Pool 2	10	6.80	0.54	7.9%
Pool 3	10	28.40	1.67	5.9%

*As measured in ten experiments in duplicate over seven days.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 µIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose:

For I hr incubation = 0.078 µIU/ml
For 2 hr incubation = 0.027 µIU/ml

14.3 Accuracy

The TSH AccuBind® ELISA test system was compared with a reference immunochemiluminescence assay. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.01µIU/ml – 61µIU/ml). The total number of such specimens was 241. The least square regression equation and the correlation coefficient were computed for the TSH AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	4.54	y = 0.47 + 0.968 (x)	0.995
Reference	4.21		

Only slight amounts of bias between the TSH AccuBind® ELISA method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the TSH AccuBind® ELISA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyrotropin needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Thyrotropin (hTSH)	1.0000	-
Follitropin (hFSH)	< 0.0001	1000ng/ml
Lutropin Hormone (hLH)	< 0.0001	1000ng/ml
Chorionic Gonadotropin(hCG)	< 0.0001	1000ng/ml

14.5 Correlation between 1 hr and 2 hr incubation

The one- (1) hr and two (2) hr (optional) incubation procedures were compared. Thirty (30) biological specimens (ranging from 0.1 – 18.5 µIU/ml) were used The least square regression equation and the correlation coefficient were computed for the 2 hr procedure (y) in comparison with the 1 hr method (x). Excellent agreement is evidenced by the correlation coefficient, slope and intercept: Y = 0.986 (x) + 0.119 Regression Correlation =0.998

15.0 REFERENCES

1. Hopton MR, & Harrap JJ, "Immunoradiometric assay of thyrotropin as a first line thyroid function test in the routine laboratory", *Clinical Chemistry,* 32, 691 (1986).
2. Caldwell, G et al, "A new strategy for thyroid function testing", *Lancet*, I, 1117 (1985).
3. Young DS, Pestaner LC, and Gilberman U, "Effects of Drugs on Clinical Laboratory Tests", *Clinical Chemistry,* 21, 3660 (1975).
4. Spencer, CA, et al, "Interlaboratory/Intermethod differences in Functional Sensitivity of Immunometric Assays of Thyrotropin (TSH) and Impact on Reliability of Measurement of Subnormal Concentrations of TSH", *Clinical Chemistry,* 41, 367 (1995).
5. Beck-Peccoz P, Persani L, "Variable biological activity of thyroid stimulating hormone", *Eur J Endocrinol,* 131, 331-340 (1994).
6. Bravermann, LE, "Evaluation of thyroid status in patients with thyrotoxicosis", *Clin Chem*, 42, 174-181 (1996).
7. Fisher, DA, "Physiological variations in thyroid hormones. Physiological and pathophysiological considerations", *Clin Chem*, 42, 135-139 (1996).

Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP325 Product Code: *325-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	C)	1 plate	2 plates	5 plates	10 plates
	D)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	E)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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IVD 2-8° CE
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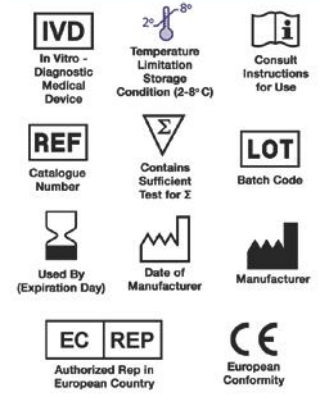
Glossary of Symbols
(EN 980/ISO 15223)



Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Free Thyroxine (Free T4) Test System

Product Code: 1225-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Free Thyroxine Concentration in Human Serum by a Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Thyroxine, the principal thyroid hormone, circulates in blood almost completely bound to carrier proteins. The main carrier is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of thyroxine is responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total thyroxine level changes so that the free thyroxine concentration remains constant. Thus, measurements of free thyroxine concentrations correlate better with clinical status than total thyroxine levels.

The increase in total thyroxine associated with pregnancy, oral contraceptives and estrogen therapy occasionally result in total T4 levels over the limits of normal while the free thyroxine concentration remains in the normal reference range. Masking of abnormal thyroid function can also occur in both hyper and hypothyroid conditions by alterations in the TBG concentration. The total T4 can be elevated or lowered by TBG changes such that the normal reference levels result. The free thyroxine concentration can help in uncovering the patient's actual clinical status.

In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T4 conjugate (analog method) is added and the reactants are mixed. A competition reaction results between the enzyme conjugate and the free thyroxine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine conjugate via a wash step. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known free thyroxine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with free thyroxine concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay, Analog Method for Free-T4 (TYPE 5):

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native free antigen, a competition reaction results between the native free antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. The interaction is illustrated by the followed equation:

$$Enz_{Ag} + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + {}^{Enz}AgAb_{C.W.}$$

$Ab_{C.W.}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$^{Enz}Ag\,Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex
K_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen con-centration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Free T4 Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of human serum based reference calibrators for free thyroxine at **approximate*** concentrations of 0 (**A**), 0.40 (**B**), 1.25 (**C**), 2.10 (**D**), 5.00 (**E**) and 7.40 (**F**) ng/dl. Store at 2-8°C. A preservative has been added. For SI units use the conversion factor 12.9 to convert ng/dl to pmol/L.
* Exact levels are given on the labels on a lot specific basis.

B. fT4- Enzyme Reagent – 13 ml/vial - Icon
One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. Free T4 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7 ml/vial - Icon S^A
One (1) bottle containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.

F. Substrate B – 7 ml/vial - Icon S^B
One (1) bottle containing hydrogen peroxide (H_2O_2) in acetate buffer. Store at 2-8°C.

G. Stop Solution – 8 ml/vial - Icon
One (1) bottle containing a strong acid (1N HCl). Store at 2-8 °C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a 96-well microplate. For other kit configurations, please refer to the table at the end of this IFU.

4.1 Materials Required But Not Provided:

1. Pipette capable of delivering 50µl & 100µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.
Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8^{0}C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20^{0}C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION:

1. **Wash Buffer**
   Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
2. **Working Substrate Solution**
   Pour the contents of the plastic vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1 : Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of fT4 Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

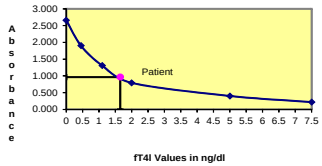
10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of free T4 in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Free T4 concentration in ng/dl on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Free T4 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/dl) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.964) intersects the dose response curve at (1.65ng/dl) free T4 concentration (See Figure 1).

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay. **Assigned values for calibrators are lot specific.**

Figure 1



EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value* (ng/dl)
Cal A	A1	2.658	2.612	0.00
	B1	2.566		
Cal B	C1	1.919	1.900	0.45
	D1	1.880		
Cal C	E1	1.339	1.306	1.10
	F1	1.273		

Cal D	G1	0.769	0.790	2.00
	H1	0.811		
Cal E	A2	0.396	0.400	5.00
	B2	0.404		
Cal F	C2	0.215	0.217	7.40
	D2	0.219		
Ctrl 1	E2	1.827	1.835	**0.50**
	F2	1.843		
Ctrl 2	G2	0.541	0.557	**2.70**
	H2	0.573		
Patient	A3	0.951	0.964	**1.65**
	B3	0.976		

Note 1: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/dl should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. If a patient, for some reason, reads higher than the highest calibrator report as such (e.g. > 7.4 ng/dl). ***Do not try to dilute the sample. TBG variations in different matrices will not allow Free T4 hormone to dilute serially.***
7. Serum free-thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, Thyroxine binding globulin (TBG) concentration, and the binding of Thyroxine to TBG (3, 4). Thus, free-Thyroxine concentration alone is not sufficient to assess the clinical status.
8. Serum free-thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives.
9. A decrease in free thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect free Thyroxine values, has been compiled by the Journal of the American Association of Clinical Chemists.
10. The interpretation of Free T4 is complicated by a variety of drugs that can affect the binding of T4 to the thyroid hormone carrier proteins or interfere in its metabolism to T3. In severe non-thyroidal illness (NTI) the assessment of thyroid becomes especially difficult. Since the patients in this category may suffer from concomitant primary hypothyroidism or from compensatory secondary hypothyroidism. In cases like these a sensitive TSH evaluation of the patient may be recommended. Please see Monobind Cat# 325-300.
11. In rare conditions associated with extreme variations in albumin binding capacity for T4- such as familial dysalbuminemic hyperthyroxinemia (FDH) – direct assessment of Free T4 may be misleading.
12. Circulating antibodies to T4 and hormone binding inhibitors may interfere in the performance of the assay.
13. Heparin is reported to have in vivo and in vitro effects on free T4 levels. Samples from patients undergoing heparin therapy should be collected well before the administration of the anticoagulant.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the Free T4 AccuBind® ELISA test system. The mean (X) values, standard deviations (σ) and expected ranges (±2σ) are presented in Table 1.

TABLE 1
Expected Values for Free T4 ELISA Test System (in ng/dl)

	Adult	Pregnancy
Number of Specimens	89	31
Mean (X)	1.40	1.50
Standard Deviation (σ)	0.30	0.37
Expected Ranges (±2 σ)	0.8 – 2.0	0.76 – 2.24

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The *inter* and *intra* assay precisions of the Free T4 AccuBind® ELISA test system were determined by analyses on three different levels of pooled patient sera. The number (n), mean values (x), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

In order to validate the *intra-assay* precision of the Free T4 AccuBind® ELISA test system, twenty replicates of each of three pooled sera (low medium and high ranges of the dose response curve) were assayed in the same assay. An intra-assay precision of 3.25 to 10.98% was obtained.

TABLE 2
Intra-Assay Precision (in ng/dl)

Sample	N	X	σ	C.V.
Low	20	0.550	0.061	10.98%
Medium	20	1.740	0.074	4.26%
High	20	3.250	0.106	3.25%

In order to validate the *inter-assay* precision of fT4 AccuBind® ELISA test system, one duplicate of each of three pooled sera (low medium and high ranges of the dose response curve) was assayed in 10 assays done over a period of six months that involved five different sets of reagents and three different technicians. An inter-assay precision of 6.01 to 10.81% was obtained.

TABLE 3
Inter-Assay Precision (in ng/dl)

Sample	N	X	σ	C.V.
Low	10	0.480	0.052	10.81%
Medium	10	1.410	0.085	6.01%
High	10	3.490	0.279	7.90%

14.2 Sensitivity

The Free T4 AccuBind® ELISA test system has a sensitivity of 0.162 ng/dl. The sensitivity was ascertained by determining the variability of the 0 ng/dl serum calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

14.3 Accuracy

The Free T4 AccuBind® ELISA test system was compared with a coated tube radioimmunoassay (**RIA)** method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.1ng/dl – 8ng/dl). The total number of such specimens was 197. The least square regression equation and the correlation coefficient were computed for this Free T4 AccuBind® ELISA method in comparison with the predicate method (Table 4).

TABLE 4
Linear Regression Analysis

Method	Mean (x)	Equation	Correlation Coefficient
Monobind EIA "X"	1.56	y = 0.1034 + 0.9525x	0.920
Predicate RIA "Y"	1.59		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values.

14.4 Specificity:

The cross-reactivity of the thyroxine antibody used for Free T4 AccuBind® ELISA to selected substances was evaluated by adding massive amounts of the interfering substance to a serum matrix. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of thyroxine needed to displace the same amount of the conjugate.

Substance	Cross Reactivity	Concentration
l–Thyroxine	1.0000	----
d-Thyroxine	0.9800	10µg/dl
d-Triiodothyronine	0.0150	100µg/dl
l–Triiodothyronine	0.0300	100µg/dl
Iodothyrosine	0.0001	100µg/ml
Diiodotyrosine	0.0001	100µg/ml
Diiodothyronine	0.0001	100µg/ml
TBG	N/D	40 µg/ml
Albumin	N/D	40 mg/ml
Phenylbutazone	N/D	10 µg/ml
Phenytoin	N/D	40 µg/ml
Salicylates	N/D	500 µg/ml

15.0 REFERENCES

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Revision: 6 **Date: 2022-MAY-01** **DCO: 1557**
Cat #: *1225-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	C)	1 plate	2 plates	5 plates	10 plates
	D)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	E)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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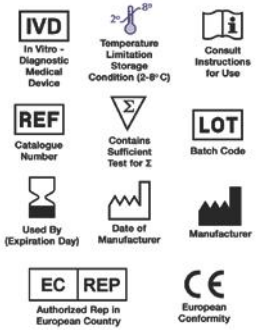
Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)



Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

D-Dimer Test System
Product Code: 12025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of D-Dimer Concentration in Human Plasma and Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

D-Dimer is the term for the cross-linked dimer of fibrinogen degradation product (FDP) D. After fibrinogen is formed in a blood clot, it is broken down through a series of steps so that it can be cleared from the body. D-Dimer is the endpoint of this process which makes its elevation a useful marker for activation of the coagulation and fibrinolytic systems.[1]
D-Dimer concentration may be determined by a blood test to help diagnose thrombosis. Since its introduction in the 1990s, it has become an important test performed in patients with suspected thrombotic disorders. While a negative result practically rules out thrombosis, a positive result may indicate thrombosis but does not definitively rule out other potential causes.[1] Its main use, therefore, is to exclude thromboembolic disease where the probability is low. Additionally, the D-Dimer test can be used in the diagnosis of disseminated intravascular coagulation.[1]
In general circumstances, a D-Dimer value under 500 ng/ml fibrin equivalence units (FEU) excludes deep vein thrombosis (DVT), pulmonary embolism (PE) and other venous thromboembolism (VTE). However, baseline D-Dimer levels increase with age and during pregnancy so modified cut-off values should be implemented for these types of patients to minimize false-positive results.[2-3]
Monitoring D-Dimer levels has become increasingly important as a positive result is indicative of an increased mortality risk. Specifically, detecting high levels of D-Dimer in cancer and pulmonary infection patients is important. VTE is the second highest cause of death in patients with cancer while significant increases in D-Dimer have been linked to higher mortality in those suffering from lung diseases such as COVID-19.[4-6]
The D-Dimer AccuBind® Test System is a quantitative test designed to be sensitive across a wide range of D-Dimer values. The reagents utilize monoclonal mouse antibodies to create a sandwich complex via a simple, fast, and user-friendly protocol.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen.

Upon mixing assay buffer and a serum containing the native antigen, reaction results between the native antigen and the coated antibody, forming an antibody-antigen complex. This interaction is illustrated below:

$$Ag + Ab_{(C)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{(C)}$$

$Ab_{(C)}$ = Coated Antibody (Excess Quantity)
Ag = Native Antigen (Variable Quantity)
$AgAb_{(c)}$ = Antigen-Antibody complex (Variable Quant.)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-coated-antibody sandwich complex on the surface of the wells.

$$AgAb_{(C)} + Ab_{(Enz)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ab_{(Enz)}AgAb_{(C)}$$

$AgAb_{(c)}$ = Antigen-Antibody complex (Variable Quant.)
$Ab_{(Enz)}$ = Enzyme-labeled Antibody (Excess Quant.)
$Ab_{(Enz)}AgAb_{(C)}$ = Sandwich complex (Variable Quant.)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

After another incubation period, the excess enzyme-labeled antibody is separated by washing. The remaining complex is then quantified by addition of substrate that reacts with bound enzyme. The amount of antigen is directly related to the amount of substrate converted by the enzyme.

4.0 REAGENTS

Materials Provided:

A. D-Dimer Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of references for D-Dimer Antigen at levels of 0(**A**), 100(**B**), 400(**C**), 1500(**D**), 4000(**E**) and 10000(**F**) ng/ml FEU. Store at 2-8°C. A preservative has been added.

B. D-Dimer Control – 1 ml/vial – Icon M
One (1) vial of reference control for D-Dimer. Store at 2-8°C. A preservative has been added.

C. Assay Buffer – 12 ml/vial - Icon Ⓑ
One (1) vial containing buffer, dye, and preservatives. Store at 2-8°C.

D. D-Dimer Enzyme Reagent – 13 ml/vial - Icon Ⓔ
One (1) vial containing Enzyme (HRP) labeled Anti-D-Dimer monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

E. D-Dimer Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with Anti-D-Dimer monoclonal mouse IgG and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

F. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

G. Substrate A – 7 ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

H. Substrate B – 7 ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

I. Stop Solution – 8 ml/vial - Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-30°C.

J. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025 and 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood plasma (EDTA, Li-Heparin, or Citrate may be used as anticoagulant) or serum in type and the usual precautions in the collection of venipuncture samples should be observed. In order to avoid erroneous results, blood samples should be centrifuged within 15 minutes of collection and the plasma or serum should be removed from the red cells immediately.

Plasma samples may be refrigerated at 2-8°C for a maximum period of three (3) days. Serum samples may be refrigerated at 2-8°C for up to fourteen (14) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05 ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution** – Stable for one year.
   Pour the contents of the amber vial labeled Substrate 'A' into the clear vial labeled Substrate 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed. (Duplicate is recommended) **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Assay Buffer to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 20 minutes at room temperature
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of D-Dimer-Enzyme Reagent to all wells.
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Incubate 20 minutes at room temperature.
10. Wash the wells three (3) times by following steps 6 and 7 as above.
11. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells**
    DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
12. Incubate at room temperature for fifteen (15) minutes.
13. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). **Always add reagents in the same order to minimize reaction time differences between wells**
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of D-Dimer in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding D-Dimer concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of D-Dimer for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.761) intersects the dose response curve at (3315 ng/ml) D-Dimer concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

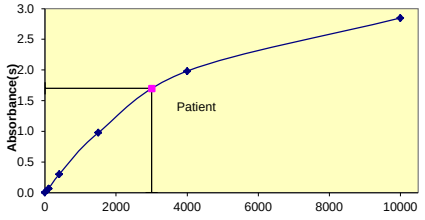
EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.005	0.004	0
	B1	0.003		
Cal B	C1	0.066	0.065	100
	D1	0.065		
Cal C	E1	0.290	0.299	400
	F1	0.308		
Cal D	G1	0.968	0.977	1500
	H1	0.986		
Cal E	A2	2.027	1.982	4000
	B2	1.938		
Cal F	C2	2.881	2.848	10000

	D2	2.815		
Ctrl 1	E2	0.094	0.098	**146**
	F2	0.101		
Ctrl 2	G2	0.951	0.965	**1476**
	H2	0.979		
Patient	A3	1.704	1.761	**3315**
	B3	1.807		

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 'F' should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with D-Dimer concentrations above 10,000 ng/ml may be diluted 1:10 with the "0" calibrator matrix or other normal serum containing low levels of D-Dimer (< 500 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor and adding the D-Dimer concentration of the diluent used.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. **A D-Dimer value alone is not of diagnostic value** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.

13.0 EXPECTED RANGES OF VALUES

The expected D-Dimer levels for exclusion of thrombosis in plasma samples were obtained from published literature.[2-3, 7] There is general consensus for the following data.

Table 1: Expected Plasma D-Dimer Levels

Patient Age	D-Dimer Level to Exclude Thrombosis
<50 years	<500 ng/ml FEU
>50 years	<Age x 10 ng/ml FEU

To obtain a reference range for serum samples, D-Dimer levels were measured by the D-Dimer AccuBind® Test System in apparently normal adults of different age groups. The values obtained are shown in Table 2.

Table 2: Serum D-Dimer Ranges

Patient Age (years)	N	Average (ng/ml FEU)	Highest (ng/ml FEU)	Lowest (ng/ml FEU)
<50	23	326	1244	137
50-59	19	486	1375	161
60-69	11	467	859	180
70-79	3	440	808	230
80+	3	878	1206	311

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISITICS

14.1 Precision

The intra-assay precision of the D-Dimer AccuBind® ELISA test system was determined by measuring sixteen (16) replicates of three levels of patient control pools on the same assay run. The results are shown in Table 3.

Table 3: Intra-assay Precision

Sample	N	Mean (ng/ml)	σ	CV%
Control 1	16	200	10.2	5.1
Control 2	16	1934	51.5	2.7
Control 3	16	4237	159.8	3.8

The inter-assay precision (total precision) of the D-Dimer AccuBind® ELISA test system was determined by measuring three levels of patient control pools on three different kits throughout the course of two months. The results are given in Table 4.

Table 4: Inter-assay Precision

Sample	N	Mean (ng/ml)	σ	CV%
Control 1	24	147	9.3	6.4
Control 2	24	1492	96.3	6.5
Control 3	24	3352	174.0	5.2

14.2 Sensitivity

The D-Dimer AccuBind® ELISA test system has a sensitivity of 4.76 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

14.3 Accuracy

14.3.1 Linearity

The linearity of the D-Dimer AccuBind® ELISA Test System was tested by serially diluting several human plasma and serum samples containing high levels of D-Dimer (up to 11,000 ng/ml) with the "0 ng/ml" serum reference. The observed values were plotted against the expected values and the test system was determined to have excellent linearity up to 11,000 ng/ml with a slope of 0.977 and a correlation factor (R^2) of 0.998.

14.3.2 Recovery

Several human plasma and serum samples containing low levels of D-Dimer (100-700 ng/ml) were spiked with 100, 400, 1200, 4000, and 8000 ng/ml of D-Dimer and assayed on the D-Dimer AccuBind® ELISA Test System. The system demonstrated excellent recovery with all observed values falling within 15% of the expected values.

14.4 Specificity

The following substances were tested on the D-Dimer AccuBind® ELISA test system to determine interference and cross-reactivity. The results are tabulated below.

Substance	Cross Reactivity	Concentration
D-Dimer	1.0000	----
Fibrogen	< 0.0001	4mg/ml
Plasminogen	< 0.0001	150ng/ml
Angiostatin	< 0.0001	150ng/ml
tPA	< 0.0001	150ng/ml
PAI1	< 0.0001	150ng/ml

15.0 REFERENCES

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Revision: 1
Date: 2021-MAR-30 DCO: 1479
MP12025 Product Code: 12025-300

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1ml	1ml
	C)	12ml	2 (12ml)
	D)	13ml	2 (13ml)
	E)	1 plate	2 plates
	F)	1 (20ml)	1 (20ml)
	G)	1 (7ml)	2 (7ml)
	H)	1 (7ml)	2 (7ml)
	I)	1 (8ml)	2 (8ml)

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Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Vitamin B12 (Vit B12) Test System
Product Code: 7625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Vitamin B12 Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Vitamin B12 is one of the nine water soluble vitamins important for healthy body functioning. The most important roles Vitamin B12 plays in the human body are in the formation of red blood cells and the formation of the myelin sheath around the nerves. Since the effects are seen in body systems with a large range of function, the symptoms of Vitamin B12 deficiency can sometimes be very ambiguous. A deficiency may also take from months to years to manifest depending on the cause and severity.[1,2,3]

Two of the most common causes of Vitamin B12 deficiency are diet and age. Because most sources of dietary Vitamin B12 come from animals, vegans who do not efficiently supplement their diet are at risk. The elderly community is also at high risk because of their diet, as well as the less efficient functioning of their digestive system.[1,3,4]

Intake of Vitamin B12 starts by ingestion and then digestion by saliva. Once reaching the gut, Vitamin B12 bound to proteins in food are released by the acids present. The B12 can then bind the Intrinsic factor. Once bound to IF, Vitamin B12 is stable enough to travel into the intestines where it can be absorbed into your body through of its association with IF.[1,5,6,7]

Two very useful tests to distinguish between Vitamin B12 deficiency and folate deficiency are methylmalonyl CoA (MMA) and homocysteine (hcy). Both deficiencies are represented by similar symptoms; however, even though both show increased levels of homocysteine, only Vitamin B12 deficiency causes an increase in methylmalonyl CoA. The increase in levels of methylmalonyl CoA and homocysteine is thought to be the root cause of any symptoms that accompany a Vitamin B12 deficiency. High levels of these two analytes in the blood stream causes increased oxidative stress to cells therefore causing increased apoptosis. In turn, vascular disease results in the form of atherosclerosis, coronary heart disease and/or neurodegeneration (ex. Parkinson's Disease).[1,8,9]

3.0 PRINCIPLE

Delayed Competitive Enzyme Immunoassay (TYPE 9):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. The interaction is illustrated by the following equation:

$$Ag + Ab_{Btn} \rightleftharpoons AgAb_{Btn}$$

Ab_{Btn} = Biotinylated antibody
Ag = Antigen (Variable Quantity) $AgAb_{Btn}$ = Immune Complex

After a short incubation, the enzyme conjugate is added (this delayed addition permits an increase in sensitivity for low concentration samples). Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binging sites (not consumed in the first incubation).

$$^{Enz}Ag + Ag + rAb_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
rAb_{Btn} = Biotinylated antibody not reacted in first incubation
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$

$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Vitamin B12 Calibrators – 1ml/vial - Icons A-F
Six (6) vials containing human serum albumin reference calibrators for Vitamin B12 at concentrations of 0 (**A**), 100 (**B**), 200 (**C**), 400 (**D**), 1000 (**E**), and 2000 (**F**) in pg/ml. A preservative has been added. Store at 2-8°C.
The calibrators can be expressed in molar concentrations (pM/L) by multiplying by 0.738. For example:
100pg/ml x 0.738= 73.8 pM/L

B. Vitamin B12 Enzyme Reagent – 7.0 ml/vial – Icon Ⓔ
One (1) vial containing Vitamin B12 (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix. Store at 2-8°C.

C. Vitamin B12 Biotin Reagent – 7.0 ml/vial – Icon ∇
One (1) vial containing anti-Vitamin B12 biotinylated purified rabbit IgG conjugate in buffer, dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20.0 ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate Reagent – 12.0 ml/vial – Icon S^N
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8.0 ml/vial – Icon STOP
One (1) vial containing a strong acid (H_2SO_4). Store at 2-8°C

H. Releasing Agent – 14.0 ml/vial – Icon Ξ
One (1) vial containing a strong base (sodium hydroxide) and potassium cyanide. Store at 2-8°C.

I. Stabilizing Agent – 0.7 ml/vial – Icon Π
One (1) vial containing tris 2-carboxyethyl)phosphine (TCEP) solution. Store at 2-8°C.

J. Neutralizing Buffer – 7.0 ml/vial – Icon NZ
One (1) vial containing buffer with dye that reduces the pH of sample extraction. Store at 2-8°C.

K. Product Insert

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate**.**

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050 & 0.100ml (50 & 100µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Glass test tubes for calibrators, control, and patient sample preparation.
5. Microplate washer or a squeeze bottle (optional).
6. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good labor-atory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirements.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube(s) with no anti-coagulants. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

2. EXTRACTION AGENT
Add an aliquot of the stabilizing agent in order to prepare a 1/40 (stabilizing agent / releasing agent) dilute solution. For example, to make 4ml (4000µl), add 0.100ml (100µl) stabilizing agent to 3.9ml (3900µl) releasing agent.

3. SAMPLE EXTRACTION (*See Note 3)*
Obtain enough test tubes for preparation of all patient samples, controls, and calibrators. Dispense 0.10ml (100µl) of all samples into individual test tubes. Pipette 0.050ml (50µl) of the prepared extraction agent to each test tube, shaking *(see note 3)* after each addition. Let the reaction proceed for 15 min. At end of the 15 min, dispense 0.050 ml (50µl) of the neutralizing buffer, vortex *(see note 3)*.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.
Note 3: Use of multiple (3) touch vortex is recommended.
Note 4: It is extremely important to accurately dispense the correct volume with a calibrated pipette and by adding near the bottom of the glass tubes at an angle while touching the side of the tubes.
Note 5: Samples with high protein concentration should be diluted 1:1 with a saline solution before performing the extraction.
Note 6: See www.monobind.com/education-center for Step-by-Step Guide on Sample Extraction for Vitamin B12 (& Folate) in Lab Tips

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50 µL) of the appropriate extracted Vitamin B12 calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of the Vitamin B12 Biotin Reagent to all wells.
4. Mix the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 45 minutes at room temperature.
6. Add 0.050 ml (50 µl) of Vitamin B12 Enzyme Reagent to all wells.
   Add directly on top the reagents dispensed in the wells.
7. Mix the microplate gently for 20-30 seconds to mix.
8. Cover and incubate for 30 minutes at room temperature.
9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
10. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
11. Add 0.100 ml (100 µl) of substrate reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
    DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
12. Incubate at room temperature for twenty (20) minutes.
13. Add 0.050 ml (50 µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 2000pg/ml 1:5 and 1:10 with Vitamin B12 '0' pg/ml calibrator and re-assay.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Vitamin B12 in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate calibrator versus the corresponding Vitamin B12 concentration in pg/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Vitamin B12 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.53) intersects the dose response curve at 391.4 pg/ml Vitamin B12 concentration (See Figure 1).

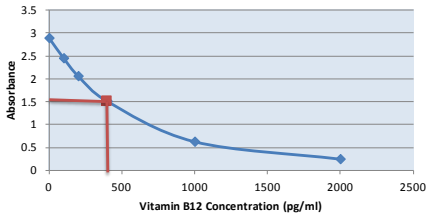
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (pg/ml)
Cal A	A1	2.898	2.89	0
	B1	2.891		
Cal B	C1	2.495	2.45	100
	D1	2.415		
Cal C	E1	2.107	2.06	200
	F1	2.023		
Cal D	G1	1.544	1.51	400
	H1	1.468		
Cal E	A2	0.662	0.63	1000
	B2	0.604		
Cal F	C2	0.263	0.25	2000
	D2	0.239		
Pat# 1	G2	1.479	1.53	391.4
	H2	1.573		

*The above data and table below is for example only. Do not use it for calculating your results.

Figure 1: Vitamin B12



Note: Multiply the horizontal values by 0.738 to convert into pM/ml.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (OD) of calibrator 0 pg/ml should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, is essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis, as required by CE Mark IVD Directive 98/79/EC, for this and other devices made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (*Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33).* For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" population the expected ranges for the Vitamin B12 AccuBind® ELISA Test System are detailed in Table 1.

TABLE 1
Expected Values - Vit B12 AccuBind® ELISA Test System[12]

Population	pg/ml	pmol/L
Newborn	160 - 1300	118-959
Adult	200 – 835	148 – 616
Adult > 60 y	110 – 800	81 – 590

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Vitamin B12 AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Low	20	334.8	24.3	7.3%
Normal	20	484.9	17.6	3.6%
High	20	925.3	28.3	3.1%

TABLE 3
Between Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Low	18	314.9	49.4	15.7%
Normal	18	441.3	46.7	10.6%
High	18	913.1	39.4	4.8%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The Vitamin B12 AccuBind® ELISA Test System has a sensitivity of 70.13 pg/ml. The sensitivity was ascertained by determining the variability of the 0 pg/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Vitamin B12 AccuBind® ELISA Test System was compared with a reference method. Biological specimens from low, normal and relatively high Vitamin B12 level populations were used (the values ranged from 156 pg/ml – 1830 pg/ml). The total number of such specimens was 56. The least square regression equation and the correlation coefficient were computed for this Vitamin B12 test in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method (Y)	654.3	y= 1.0186x -48.82	0.9506
Reference (X)	690.2		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross reactivity of the Vitamin B12 antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Vitamin B12 needed to displace the same amount of labeled analog.

TABLE 5

Substance	Cross Reactivity
Bilirubin	0.0003
Rhematoid Factor	0.0008
Cobinamide	<0.0001
Lipemia	<0.0001
Hemoglobin	<0.0001

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Effective Date: 2019-Jul-16 Rev. 6 **DCO: 1353**
MP7625 **Product Code: 7625-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (7ml)	2 (7ml)
	C)	1 (7ml)	2 (7ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	1 (8ml)
	H)	1 (14ml)	2 (14ml)
	I)	1 (0.7ml)	2 (0.7ml)
	J)	1 (7ml)	2 (7ml)

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IVD 2°-8° CE
EC REP CEpartner4U, Esdoornlaan 13 3951 DBMaarn, The Neatherlands www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2°-8° Temperature Limitation Storage Condition (2-8° C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE European Conformity	

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

25-OH Vitamin D Total (Vit D-Direct) Test System

Product Code: 9425-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of 25-OH Vitamin D Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Vitamin D is a fat soluble secosteroid hormone that is important in the management of calcium and phosphorus concentrations required in the mineralization of bone. Vitamin D has two important forms: cholecalciferol (D_3) formed in the skin from ultraviolet light and ergocalciferol (D_2) found in dairy products. However, these forms do not have significant biological activity. The hormonal active form, 1, 25-dihydroxylcholecalciferol, is produced through transformations in the liver and kidney. The first step in this conversion is an enzymatic reaction of D_2 or D_3 into 25OH-D_2 or 25OH-D_3. These 25OH D forms are not freely circulating in blood, but are primarily bound to vitamin D binding protein (VDBP). The high binding affinity of the 25OH $D_{(2 \text{ or } 3)}$ compared to other derivatives of vitamin D leads to a long half-life in blood and its use as an accurate indicator of Vitamin D status. Vitamin D deficiency has been associated to diseases related to bone damage such as osteomalacia and rickets. Vitamin D can be dietarily supplemented through the use of Vitamin D_2 or vitamin D_3. The sum of the 25OH $D_{(2 \text{ and } 3)}$ in serum or plasma is referred to as total 25OH Vitamin D. The accurate measurement of total vitamin D is necessary in monitoring deficient vitamin D patients to achieve the optimum dosage and avoid excessive levels, which are considered toxic.

3.0 PRINCIPLE

Sequential Competitive Method (Type 6):
The essential reagents required for a solid phase sequential enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, and a serum sample containing the native antigen, a binding reaction results between the native antigen for a limited number of insolubilized binding sites. The interaction is illustrated by the following equation:

$$Ag + Ab_{CW} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{CW}$$

Ab_{CW} = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
$AgAb_{CW}$ = Antigen-Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

After removing any unreacted native antigen by a wash step, the enzyme-conjugated antigen is introduced. The conjugate reacts with sites of the antibody unoccupied by the native antigen.

$$^{Enz}Ag + Ab_{CW} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}AgAb_{CW}$$

^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$^{Enz}AgAb_{CW}$ = Enzyme-antigen Conjugate-Antibody Complex

After a short second incubation, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different calibrators of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Vit D Calibrators – 1ml/vial – Icons A-G
Seven (7) vials containing human serum albumin reference calibrators for 25-OH Vitamin D at concentrations of 0 (**A**), 5 (**B**), 10 (**C**), 25 (**D**), 46 (**E**), 85 (**F**), and 150 (**G**) in ng/ml. A preservative has been added. Store at 2-8°C.
The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 2.5. For example:
10ng/ml x 2.5 = 25nM/L

B. Vit D Controls – 1ml/vial – Icons M-N
Two (2) vials containing human serum reference controls at concentration established (exact value listed on label). A preservative has been added. Store at 2-8°C.

C. Vit D Releasing Agent – 12 ml/vial – Icon
One (1) vial containing vitamin D binding protein releasing agents. Store at 2-8°C.

D. Vit D Enzyme Reagent – 12 ml/vial – Icon
One (1) vial containing 25-OH Vitamin D_3 (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix. Store at 2-8°C.

E. Vit D Antibody Coated Plate – 96 wells – Icon
One 96-well microplate coated with < 1.0 µg/ml anti-Vitamin D sheep IgG and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

F. Wash Solution Concentrate – 20 ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

G. Substrate Reagent – 12 ml/vial – Icon S^N
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8 ml/vial – Icon STOP
One (1) vial containing a strong acid (H_2SO_4). Store at 2-8°C

I. Product Insert

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 & 0.100ml (25 & 100µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050, 0.100 & 0.350ml (50, 100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirements.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube(s) with no anti-coagulants. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25 µL) of the appropriate extracted 25-OH Vitamin D calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100 µl) of the 25-OH Vitamin D Releasing Agent to all wells.
4. Mix **(Note 3)** the microplate for 20-30 seconds until homogeneous.
5. Cover and incubate for 30 minutes at room temperature
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100 µl) of 25-OH Vitamin D Enzyme Reagent to all wells.

DO NOT SHAKE THE PLATE AFTER ADDITION

9. Cover and incubate for 30 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100 µl) of substrate reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE (MIX) THE PLATE AFTER SUBSTRATE ADDITION

13. Incubate at room temperature for twenty (20) minutes.
14. Add 0.050 ml (50 µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note1: **Do not use the substrate reagent if it looks blue.**
Note 2: **Do not use reagents that are contaminated or have bacteria growth.**
Note 3: **Cycle (start and stop) mixing (4 cycles) for 5-8 seconds/cycle is more efficient than one continuous (20-30 seconds) cycle to achieve homogeneity. A plate mixer can be used to perform the mixing cycles.**
Note 4: **It is extremely important to accurately dispense the correct volume with a calibrated pipette and by adding near the bottom of the microwells at an angle while touching the side of the well.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of 25-OH Vitamin D in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate calibrator versus the corresponding 25-OH Vitamin D concentration in ng/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of 25-OH Vitamin D for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.033) intersects the dose response curve at 39.9 ng/ml 25-OH Vitamin D concentration (See Figure 1).

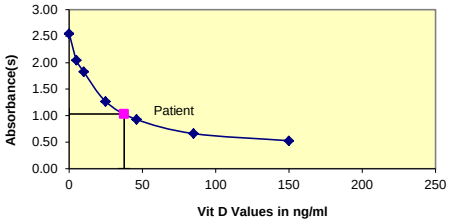
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.559	2.548	0
	B1	2.537		
Cal B	C1	2.041	2.047	5
	D1	2.054		
Cal C	E1	1.848	1.826	10
	F1	1.804		
Cal D	G1	1.286	1.267	25
	H1	1.249		
Cal E	A2	0.934	0.930	46
	B2	0.927		
Cal F	C2	0.654	0.663	85
	D2	0.712		
Cal G	G2	0.511	0.529	150
	H2	0.546		
Pat# 1	A3	1.027	1.033	37.5
	A4	1.039		

*The above data and figure below is for example only. Do not use utilize it for calculating results.

Figure 1



Note: Multiply the horizontal values by 2.5 to convert into nM/ml.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator A (0 ng/ml) should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, is essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis, as required by CE Mark IVD Directive 98/79/EC, for this and other devices made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (*Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33).* For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

Based on the published literature the following ranges have been assigned. **These ranges should be used as guidelines only:**

TABLE 1
Expected Values for the Vit D-Direct ELISA

LEVEL	RANGE (ng/ml)
Very severe vitamin D deficiency	< 5
Severe vitamin D deficiency	5-10
Vitamin D deficiency	10-20
Suboptimal vitamin D provision	20-30
Optimal vitamin D level	30-50
Upper norm	50-70
Overdose, but not toxic	70-150
Vitamin D intoxication	> 150

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the 25-OH Vitamin D AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision

Serum	N	X	σ	%C.V.
1	20	22.16	1.35	6.10
2	20	34.96	1.44	4.11
3	20	86.09	6.37	7.40

TABLE 3
Between Assay Precision

Serum	N	X	σ	%C.V.
1	45	23.88	2.14	8.96
2	45	37.53	3.44	9.17
3	45	87.91	7.1	8.08

14.2 Sensitivity

The sensitivity of the Vit-D Direct AccuBind® ELISA test system method was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The test system has an analytical sensitivity of 1.14 ng/ml Vitamin D.

14.3 Accuracy

The Vit D AccuBind® ELISA Test System was compared with a reference method. A total of 83 biological specimens from low, normal, and high Vit D level populations were used; the values ranged from 9.5ng/ml to 200ng/ml. The least square regression equation and the correlation coefficient were computed for the AccuBind method when compared to the reference method. The data obtained is displayed in Table 4.

Method	Mean	Leas Square Regression Analysis	Correlation Coefficient
Monobind (y)	52.08	y=1.02(x)+1.33	0.918
Reference (x)	49.98		

14.4 Specificity

The % cross-reactivity of the 25-OH Vitamin D antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of 25-OH Vitamin D needed to displace the same amount of labeled analog.

TABLE 5

Substance	Cross Reactivity
25-OH Vitamin D2	1.0000
25-OH Vitamin D3	1.0000
Vitamin D2	0.0076
Vitamin D3	0.0039
D2 Active 1,3,25-Hydroxy Vitamin D 2	1.9000
D3 Active 1,3,25-Hydroxy Vitamin D 3	1.1500

15.0 REFERENCES

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2. Morris H. A. "Vitamin D: A Hormone for All Seasons-How Much is enough?" *Clin. Biochem*. Rev., 2005, 26, 21-32.
3. Bikle D. D. "Vitamin D and the skin". *J. Bone Miner. Metab.*, 2010, 28, 117-30.
4. Zerwekh J. E. "Blood biomarkers of vitamin D status". *Am. J. Clin. Nutr*. 2008, 87, 1087S-91S.
5. Moyad M. A. "Vitamin D: a rapid review". *Dermatol Nurs.*, 2009, 21, 25-30.

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MP9425 **Product Code: 9425-300**

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2(2ml set)
	B)	1ml set	1ml set	2ml set	2(2ml set)
	C)	1 (12ml)	2 (12ml)	1 (60ml)	2 (60ml)
	D)	1 (12ml)	2 (12ml)	1 (60ml)	2 (60ml)
	E)	1 plate	2 plate	5 plate	10 plate
	F)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	G)	1 (12ml)	2 (12ml)	1 (52ml)	2 (52ml)
	H)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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Glossary of Symbols
(EN 980/ISO 15223)