Foresight®

Free T4 EIA Test Kit
Package Insert

REF I231-3031	English

An enzyme immunoassay (EIA) for the quantitative detection of Free T4 (Free Thyroxine) in human serum.
For professional in vitro diagnostic use only.

INTENDED USE

The Free T4 EIA Test Kit is an enzyme immunoassay for the quantitative detection of Free Thyroxine (FT4) in human serum. It is intended as an aid in the assessment and diagnosis of thyroid or pituitary disorders as well as in the follow-up of patients undergoing therapy.

SUMMARY

Thyroxine (T4) or 3,5,3',5'-tetraiodothyronine is a hormone with a molecular weight of 777 daltons secreted by the thyroid gland. T4 along with the other thyroid hormone T3 are responsible for regulating metabolism in the body. T4 circulates the blood primarily bound to carrier proteins. The main carrier protein is thyroxine binding protein (TBG) followed by thyroxine binding prealbumin (TBPA) and albumin.[2] The binding of T4 by these proteins is such that approximately 0.03% is unbound or free T4. This small fraction of T4 represents the biologically active hormone.[3,4] T4 concentrations is dependent on the level of TBG as well as patient's thyroid status during many clinical conditions such as pregnancy and administration of certain drugs. On the other hand, Free T4 is less sensitive to changes in these carrier proteins. Therefore, measurement of free T4 may provide better indication of thyroid status. The Free T4 EIA Test Kit is an immunoassay for the quantitative detection of the presence of Free T4 (Thyroxine) in serum specimen. The test utilizes monoclonal antibodies to selectively detect Free T4 in serum.

PRINCIPLE

The Free T4 EIA Test Kit is a solid phase enzyme immunoassay based on a competitive principle for the quantitative detection of Free T4 in human serum. The microwell plate is coated with a fixed amount of monoclonal antibodies specific to T4. During testing, the specimen along with T4 enzyme-conjugate are added to the antibody coated microwell plate and then incubated. If the specimen contains T4, it will compete with the T4 enzyme-conjugate to bind to the antibodies coated on the microwell plate. If the specimen does not contain Free T4, only the T4 enzyme-conjugate will bind to the inside of the plate. After initial incubation, the microwell plate is washed to remove unbound materials. Substrate A and Substrate B are added and then incubated to produce a blue color, indicating the amount of T4 enzyme-conjugate bound to the plate. If Free T4 is present in the sample, it will block the antibody binding sites and when the substrate is added there will be no color development. The absence of color or low amount of color thus indicates the presence of Free T4 in the specimen. Sulfuric acid solution is added to the microwell plate to stop the reaction which produces a color change from blue to yellow. The color intensity, which is inversely proportional to the amount of Free T4 present in the specimen, is measured with a microplate reader at 450/630-700 nm or 450 nm. The absorbance of the specimen is then compared to a calibration curve to obtain the amount of Free T4 present in the specimen.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Add all the calibrators, controls, and specimens into the wells within 15 minutes to minimize the change in absorbance which may affect the results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

HEALTH AND SAFETY INFORMATION

- Human specimens should be considered potentially hazardous and handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Substrate and Calibrators. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not pipette by mouth.
- Avoid any contact of the Substrate and Stop Solution with skin or mucosa. The Stop Solution contains 0.5 M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The Free T4 EIA Test Kit can be performed using only human serum collected from venipuncture whole blood.
- Plasma is not recommended as the testing specimen. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Serum specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS AND COMPONENTS

Materials Provided

No.	Reagent	Component Description	Quantity	
			96 wells/kit	480 wells/kit
	Free T4 Microwell Plate	Microwell plate coated with monoclonal Anti-T4	1 plate (96 wells/plate)	5 plates (96 wells/plate)
1	Conjugate (11x)	T4 bound to peroxidase; Preservative:0.1% ProClin™ 300	1 x 1.2 mL	5 x 1.2 mL
1A	Conjugate Diluent	Tris-HCl Buffer; Preservative: 0.1% ProClin™300	1 x 12 mL	5 x 12 mL
2	Concentrated Wash Buffer (25x)	Tris-HCl buffer containing 0.1% Tween 20; Preservative: 0.1% ProClin™ 300	1 x 40 mL	5 x 40 mL
3	Substrate A	Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
4	Substrate B	Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
5	Stop Solution	0.5 M Sulfuric acid	1 x 8 mL	5 x 8 mL
6	Free T4 Calibrator 1	Buffer containing BSA. Free thyroxine of reference calibrators is at **approximate** concentration of 0 ng/dL ; Preservative: 0.1% ProClin™300	1 x 0.5 mL	5 x 0.5 mL
7	Free T4 Calibrator 2	Buffer containing BSA . Free thyroxine of reference calibrators is at **approximate** concentration of 0.45 ng/dL ; Preservative: 0.1% ProClin™300	1 x 0.5 mL	5 x 0.5 mL
8	Free T4 Calibrator 3	Buffer containing BSA . Free thyroxine of reference calibrators is at **approximate** concentration of 1.25 ng/dL ; Preservative: 0.1% ProClin™300	1 x 0.5 mL	5 x 0.5 mL
9	Free T4 Calibrator 4	Buffer containing BSA . Free thyroxine of reference calibrators is at **approximate** concentration of 2.1 ng/dL ; Preservative: 0.1% ProClin™300	1 x 0.5 mL	5 x 0.5 mL
10	Free T4 Calibrator 5	Buffer containing BSA . Free thyroxine of reference calibrators is at **approximate** concentration of 5.0 ng/dL ; Preservative: 0.1% ProClin™ 300	1 x 0.5 mL	5 x 0.5 mL
11	Free T4 Calibrator 6	Buffer containing BSA . Free thyroxine of reference calibrators is at **approximate** concentration of 7.4 ng/dL ; Preservative: 0.1% ProClin™ 300	1 x 0.5 mL	5 x 0.5 mL
	Plate Sealers		2	10
	Package Insert		1	1

Materials Required But Not Provided

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 20°C to 40°C.
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Automated processor (optional)
- Calibrated micropipettes with disposable tips capable of dispensing 25, 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Timer

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the calibrators in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

Step	Detailed Procedure	Simplified Procedure
	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill it with freshly distilled or deionized water to 1000 mL for 96 wells/plate testing. The Working Wash Buffer is stable for 2 weeks at 15-30°C. **Note:** If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve. • Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 • Remove and store unused strips at 2-8°C
	• Prepare Working Conjugate Solution by diluting Conjugate with Conjugate Diluent 1:11, This dilution procedure should be done just before the test. **Note:** Conjugate diluent may have a little precipitation, please mix well before use.	• Prepare Working Conjugate Solution by diluting Conjugate with Conjugate Diluent 1:11
0	• Leave A1 as Blank well.	• Leave A1 as Blank well
1	• Add 25 µL of Calibrator 1 in wells B1 and C1. • Add 25 µL of Calibrator 2 in wells D1 and E1. • Add 25 µL of Calibrator 3 in wells F1 and G1. • Add 25 µL of Calibrator 4 in wells H1 and A2. • Add 25 uL of Calibrator 5 in wells B2 and C2. • Add 25 µL of Calibrator 6 in wells D2 and E2.	• B1 and C1: Add 25 µL Calibrator 1 • D1 and E1: Add 25 µL Calibrator 2 • F1 and G1: Add 25 µL Calibrator 3 • H1 and A2: Add 25 µL Calibrator 4 • B2 and C2: Add 25 µL Calibrator 5 • D2 and E2: Add 25 µL Calibrator 6

2	• Add 25 µL of specimen to assigned wells starting at F2 and G2.	• Starting F2 and G2: Add 25 µL specimen
3	• Add 100 µL of Working Conjugate Solution to each well except for the Blank well. (Red Reagent)	• Add 100 µL of Working Conjugate to each well
4	• Mix gently by swirling the microwell plate on a flat bench for 30 seconds. • Cover the microwell plate with the Plate Sealer and incubate at 37°C for 60 minutes ± 5minute.	• Mix gently • Cover the microwell plate with the Plate Sealer and incubate at 37°C for 60 min
5	• Remove the Plate Sealer. • Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid. • Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false results.	• Remove the Plate Sealer • Wash each well 5 times with 350 µL of Working Wash Buffer • Turn the microwell plate upside down on absorbent tissue
6	• Add 50 µL of Substrate A to each well. (Clear Reagent) • Add 50 µL of Substrate B to each well. (Clear Reagent) Then a blue to light blue color should develop in wells corresponding to the amount of Free T4 present in the specimen.	• Add 50 µL of Substrate A to each well • Add 50 µL of Substrate B to each well
7	• Mix gently then cover microwell plate with Plate Sealer and incubate at room temperature (20-30°C) for 15 minutes ± 1 minute.	• Mix then cover microwell plate with Plate Sealer and incubate at room temperature for 15 min
8	• Remove the Plate Sealer. • Add 50 µL of Stop Solution to each well. (Clear Reagent). Then a yellow to light yellow color should develop in wells corresponding to the amount of Free T4 present in the specimen.	• Remove Plate Sealer • Add 50 µL of Stop Solution to each well
9	• Read at 450/630-700 nm within 30 minutes. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.	• Read at 450/630-700 nm within 30 min

AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

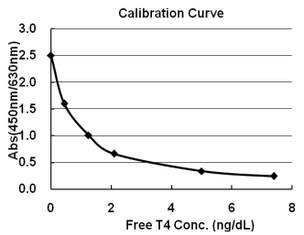
QUALITY CONTROL

Control standards are not supplied with this kit; however, it is recommended that low, middle and high titer controls be tested with each run as a good laboratory practice to monitor assay performance. Each laboratory should establish its own criteria for establishing mean values and acceptable ranges to determine reliability of the results.

CALCULATION OF RESULTS

Draw the calibration curve and obtain quantitative specimen results.

1. Record the absorbance obtained from the printout of microplate reader as outlined in the Example of Specimen & Calibrators Result Calculation.
2. Plot the absorbance for each duplicate referenceversus the corresponding concentration in ng/dL on linear graph paper.
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 onthe curve, and read the concentration (in ng/dL) from the horizontal axis of the graph. In the following example, the average absorbance intersects the dose response curve at Free T4 concentration.



Example of Specimen & Calibrators Result Calculation

Item	Well	Absorbance	Mean (Absorbance – Blank)	Concentration (ng /dL)
Blank Well	A1	0.002	/	/
Calibrator 1	B1	2.494	2.502	0.00
	C1	2.513		
Calibrator 2	D1	1.595	1.599	0.45
	E1	1.607		
Calibrator 3	F1	1.017	1.009	1.25
	G1	1.006		
Calibrator 4	H1	0.647	0.661	2.10
	A2	0.679		
Calibrator 5	B2	0.335	0.335	5.00
	C2	0.338		
Calibrator 6	D2	0.236	0.239	7.40
	E2	0.245		
Specimen	F2	0.878	0.877	1.57
	G2	0.880		

LIMITATIONS

1. The Free T4 EIA Test Kit is used for the detection of free thyroxine in human serum. Diagnosis should not be established based on a single test result. Further testing should be performed in assessing clinical status. Specimens containing precipitate may give inconsistent test results.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. As with other sensitive immunoassays, there is the possibility that the positive result cannot be repeated due to inadequate washing from the initial test. The results may be affected due to procedural or instrument error.
4. Unusually high titers of heterophile antibodies or rheumatoid factor (RF) may affect results.
5. The Free T4 EIA Test Kit is not intended for use in screening of newborns
6. The test cannot be used in patients receiving treatment with lipid-lowering agents containing D-T4.

EXPECTED VALUES

It is recommended that each laboratory establish its own range of expected values based on patient populations. A study to determine expected values using the Free T4 EIA Test Kit was conducted for initial reference use only.

Population	No. Specimens	Mean (ng/dL)	Range (ng/dL)
Normal	149	1.37	0.54-2.21

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The analytical sensitivity of the Free T4 EIA Test Kit is 0.06 ng/dL.

Accuracy

The Free T4 EIA Test Kit has been compared with electrochemiluminescence immunoassay "ECLIA" method. A total of 218 clinical specimens ranging from 0.06-7.47 ng/dL were run and analyzed using least square regression analysis. The results show that the Free T4 EIA Test Kit has good correlation compared to the reference method.

Method	Equation	Correlation
Acon EIA "X"	y = 1.002x - 0.025	0.966
Reference "Y"		

Reproducibility

Intra-Assay: Within-run precision has been determined by using 10 replicates of one specimen with the normal level of Free T4.

Inter-Assay: Between-run precision has been determined by two independent assays on the same specimen. Three different lots of the Free T4 EIA Test Kit have been tested using the specimen.

Coefficient of Variation (%)	
Intra-Assay	Inter-Assay
＜10%	＜15%

Cross-Reactivity

The specificity of the Free T4 EIA Test Kit was determined by testing sera containing the compounds listed below. These compounds showed less than 15% interference in the Free T4 EIA Test Kit at the levels indicated.

Substance	Concentration	Substance	Concentration
TBG	40 µg/mL	Sodium Salicylate	500 µg/mL
Bilirubin	200 µg/mL	Interlipid	1.2 mg/mL
Albumin	50 mg/mL	L-Triiodothyronine	100µg/dL
L-Tyrosine	750 mg/mL	E2	2000 pmol/L
Acetaminophen	350 µg/mL	E3	15 pmol/L

BIBLIOGRAPHY

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3. Robbins, J, Rall, JE. Thyroxine-Binding Proteins. In:Gray CH, Bacharach AL, editors. Hormones in Blood. Vol 1. (2nd edition) London: Academic Press (1967), 427-40.
4. Ekins RP. Free Thyroid Hormones. Amsterdam: Excerpta Medica Foundation. (1979), 72: 106

Index of Symbols

	Consult instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by	EC REP	Authorized Representative
2°C 8°C	Store between 2-8°C	LOT	Lot Number	REF	Catalog #
Conjugate Diluent	Conjugate Diluent	Free T4	Free T4	Microwell Plate	Microwell Plate
Substrate A	Substrate A	Conjugate 11x	Conjugate (11x)	Wash Buffer 25x	Wash Buffer (25x)
Plate Sealer	Plate Sealer	Substrate B	Substrate B	Stop Solution	Stop Solution
Calibrator 2	Calibrator 2	Package Insert	Package Insert	Calibrator 1	Calibrator 1
Calibrator 5	Calibrator 5	Calibrator 3	Calibrator 3	Calibrator 4	Calibrator 4
		Calibrator 6	Calibrator 6		

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