Foresight®

TSH EIA Test Kit
Package Insert

REF	I231-3011	English

An enzyme immunoassay (EIA) for the quantitative detection of TSH (Thyroid Stimulating Hormone) in human serum or plasma.

For professional in vitro diagnostic use only.

INTENDED USE

The TSH EIA Test Kit is an enzyme immunoassay for the quantitative detection of Thyroid Stimulating Hormone (TSH) in human serum or plasma. 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

Thyroid stimulating hormone (TSH), or thyrotropin is a glycoprotein with a molecular weight of approximately 28,000 daltons produced by the anterior pituitary.[1] TSH regulates the production and release of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3) from the thyroid gland which are responsible for regulating metabolism in the body. Release of TSH is regulated by T3 and T4 via negative feedback mechanism and by thyrotropin releasing hormone (TRH) which is secreted in the hypothalamus. Determining TSH levels in the blood are helpful in the differential diagnosis of primary and secondary hypothyroidism and hyperthyroidism, especially when used with the measurement of thyroid hormones.[2,3,4,5] The TSH EIA Test Kit is an immunoassay for the quantitative detection of the presence of Thyroid Stimulating Hormone (TSH) in serum or plasma specimen. The test utilizes monoclonal antibodies to selectively detect TSH in serum or plasma.

PRINCIPLE

The TSH EIA Test Kit is a solid phase enzyme immunoassay based on a sandwich principle for the quantitative detection of TSH in human serum or plasma. The microwell plate is coated with monoclonal antibodies specific to TSH. During testing, the specimen and the enzyme-conjugated TSH antibodies are added to the antibody coated microwell plate and then incubated. If the specimen contains TSH, it will bind to the antibodies coated on the microwell plate and simultaneously bind to the conjugate to form immobilized antibody-TSH-conjugate complexes. If the specimen does not contain TSH, the complexes will not be formed. 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 TSH present 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 corresponds to the amount of TSH 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 TSH 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.
- 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

- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be 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 0.5M 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 TSH EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma 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 and plasma 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
	TSH Microwell Plate	Microwell plate coated with monoclonal Anti-TSH	1 plate (96 wells/plate)	5 plates (96 wells/plate)
1	TSH Conjugate	Anti-TSH bound to peroxidase; 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	0.5M Sulfuric acid	1 x 8 mL	5 x 8 mL
6	TSH Calibrator 1	PBS buffer Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
7	TSH Calibrator 2	Buffer containing 0.5 µIU/mL TSH; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
8	TSH Calibrator 3	Buffer containing 2.5 µIU/mL TSH; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
9	TSH Calibrator 4	Buffer containing 5.0 µIU/mL TSH; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
10	TSH Calibrator 5	Buffer containing 10.0 µIU/mLTSH; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
11	TSH Calibrator 6	Buffer containing 20.0 µIU/mL TSH; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 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 37°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 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
0	• Leave A1 as Blank well.	• Leave A1 as Blank well.
1	• Add 50 µL of Calibrator 1 in wells B1and C1. • Add 50 µL of Calibrator 2 in wells D1and E1. • Add 50 µL of Calibrator 3 in wells F1and G1. • Add 50 µL of Calibrator 4 in wells H1and A2. • Add 50 µL of Calibrator 5 in wells B2and C2. • Add 50 µL of Calibrator 6 in wells D2and E2. • The colors of Calibrators 1-6 gradually change from colorless to blue. **Note:** For manual pipetting, it is recommended that no more than 32 wells be used for each run or pipetting should be completed within 5 minutes for all calibrators, specimen, and controls.	• B1and C1: Add 50 µL Calibrator 1 • D1and E1: Add 50 µL Calibrator 2 • F1and G1: Add 50 µL Calibrator 3 • H1and A2: Add 50 µL Calibrator 4 • B2and C2: Add 50 µL Calibrator 5 • D2and E2: Add 50 µL Calibrator 6
2	• Add 50 µL of specimen to assigned wells starting at F2 and G2.	• Starting F2 and G2: Add 50 µL specimen
3	• Add 100 µL of Conjugate to each well except for the Blank well. (Red Reagent)	• Add 100 µL of 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 in a water bath or an incubator for 60 minutes ± 2 minutes.	• Mix gently • Cover the microwell plate 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 positive 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 light blue to blue color should develop in wells corresponding to the amount of TSH 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) in a room, a water bath, or an incubator for 20 minutes ± 1 minute.	• Mix then cover microwell plate with Plate Sealer and incubate at room temperature for 20 min
8	• Remove the Plate Sealer. • Add 50 µL of Stop Solution to each well. (Clear Reagent)	• 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.	• Leia a 450/630-700 nm nos 30 minutos seguintes

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.

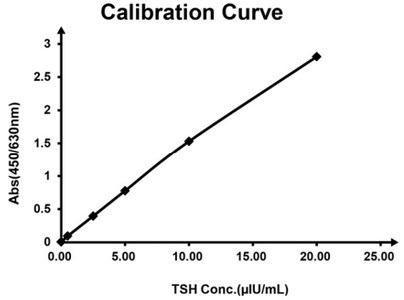
QUALITY CONTROL

Control standards are not supplied with this kit; however, it is recommended that low, normal and high 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 reference versus the corresponding concentration in µIU/mL on linear graph paper
3. Connect the points with a best-fit curve.
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. In the following example, the average absorbance intersects the dose response curve at TSH concentration.



Example of Specimen & Calibrators Result Calculation

Item	Well	Absorbance	Mean (Absorbance – Blank)	TSH Concentration (µIU/mL)
Blank Well	A1	0.003	/	/
Calibrator 1	B1	0.004	0.002	0
	C1	0.005		
Calibrator 2	D1	0.098	0.089	0.5
	E1	0.086		
Calibrator 3	F1	0.406	0.391	2.5
	G1	0.381		
Calibrator 4	H1	0.779	0.773	5
	A2	0.772		
Calibrator 5	B2	1.535	1.526	10
	C2	1.522		
Calibrator 6	D2	2.843	2.462	20
	E2	2.087		
Specimen	F2	0.405	0.410	2.62
	G2	0.420		

LIMITATIONS

1. The TSH EIA Test Kit is used for the detection of TSH in human serum or plasma. 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. Even if test results are positive, further clinical evaluation should be considered with other clinical information available to the physician.
5. The amount of TSH in serum depends upon multiple factors including the function of the hypothalamus and thyroid gland as well as the responsiveness of pituitary to TRH. TSH concentration alone is not sufficient to assess clinical status.
6. Elevated TSH values may occur during pharmacological intervention in drugs such as domperiodone, amiodazon, iodide, phenobarbital, and phenytoin, while decreased TSH values have been reported with administration of propanolol, methimazol, dopamine, and d-thyroxine.
7. The TSH EIA Test Kit is not intended for use in screening of newborns.

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 TSH EIA Test Kit was conducted for initial reference use only.

Population	No. Specimens	Mean (µIU/mL)	Range (µIU/mL)
Normal	440	1.571	0.25-5.0

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The analytical sensitivity of the TSH EIA Test Kit is 0.078 µIU/mL.

Accuracy

The TSH EIA Test Kit has been compared to a leading commercial TSH EIA test using clinical specimens. A total of 212 clinical specimens ranging from 0.008-19.350 µIU/mL were run and analyzed using least square regression analysis. The results show that the TSH EIA Test Kit has good correlation compared to the reference method.

Method	Equation	Correlation
Acon "x" Reference "y"	y=0.925x+0.420	0.959

Reproducibility

Intra-Assay: Within-run precision has been determined by using 20 replicates of one hypothyroid serum.

Inter-Assay: Between-run precision has been determined by 3 independent assays on one hypothyroid serum. Three different lots of the TSH EIA Test Kit have been tested using these specimens over a 3-day period.

Intra-Assay			Inter-Assay		
Mean (µIU/mL)	Standard Deviation	Coefficient of Variation (%)	Mean (µIU/mL)	Standard Deviation	Coefficient of Variation (%)
8.846	0.427	4.827	8.740	0.789	9.027%

Recovery and Linearity

Recovery: Known amounts of TSH were added to normal human serum with endogenous TSH concentration of 0.58 µIU/mL. The concentration of TSH was determined using TSH EIA Test Kit and the resulting percent recovery was calculated.

Specimen	TSH Concentration Added (µIU/mL)	TSH Concentration Obtained (µIU/mL)	Recovery* (%)
Level 1	1.96	1.89	96.43
Level 2	2.59	2.70	104.25
Level 3	5.64	5.37	95.21
Level 4	10.97	10.05	91.61
Level 5	18.73	18.88	102.44

* Recovery = (Concentration Obtained (µIU/mL) – Endogenous Level (µIU/mL))/Concentration Added (µIU/mL)

Linearity: Specimens containing known concentration of TSH were diluted with normal human serum and determined. The obtained concentrations were within ±20% of the expected values.

Cross-Reactivity

The specificity of the TSH EIA Test Kit was determined by testing sera containing the compounds listed below. These compounds showed less than 20% interference in the TSH EIA Test Kit at the levels indicated.

Substance	Concentration	Substance	Concentration
Uric cid	0.09 mg/mL	Bilirubin	0.15 mg/mL
Vitamin	19.80 mg/mL	EDTA	0.20 mg/mL
Globin	0.99 mg/mL	Hemoglobin	16.67 mg/mL
Gentistic cid	0.20 mg/mL	Creatin	0.20 mg/mL
Acetaminophen	0.20 mg/mL	Cyclophosphamide	0.50 mg/mL
Oxalic cid	0.99 mg/mL	5-fluorouracil	2.00 mg/mL
Albumin	20.00 mg/mL	Cytosine arabinoside	0.30 mg/mL
Coffein	0.10 mg/mL		

The following substances and concentrations have also been tested using TSH EIA Test Kit and no interference was observed.

Substance	Concentration
Follitropin (FSH)	500 mIU/mL
Lutropin Hormone (LH)	500 mIU/mL
Chorionic Gonadotropin (hCG)	500 mIU/mL

Dose Hook Effect

No dose hook effect is observed up to 562 µIU/mL of TSH.

BIBLIOGRAPHY

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Index of Symbols

	Consult instructions for use	Σ	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 #
		Substrate A	Substrate A	Substrate B	Substrate B
TSH	TSH	Conjugate	Conjugate	Calibrator 1	Calibrator 1
Wash Buffer 25x	Wash Buffer (25x)	Calibrator 3	Calibrator 3	Calibrator 4	Calibrator 4
Calibrator 2	Calibrator 2	Calibrator 6	Calibrator 6		
Calibrator 5	Calibrator 5	Stop Solution	Stop Solution	Package Insert	Package Insert
Microwell Plate	Microwell Plate	Plate Sealer	Plate Sealer		

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