






# TSH (Thyroid Stimulating Hormone) GENLISA™ ELISA

**REF** : KBD403

Ver 1.0


**IVD**

ELISA immunoassay for the Quantitative Determination of TSH  
(Thyroid Stimulating Hormone) in human serum and plasma.

<b>IVD</b>	<b>For In-vitro Diagnostic Use</b>	<b>REF</b>	<b>Catalog Number</b>
	<b>Store At</b>	<b>LOT</b>	<b>Batch Code</b>
	<b>Manufactured By</b>		<b>Biological Risk</b>
	<b>Expiry Date</b>		<b>Consult Operating Instructions</b>

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**REF** KBD403

 96 tests

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### Introduction:

Thyroid stimulating hormone is produced and released into the bloodstream by the pituitary gland. It controls production of the thyroid hormones, thyroxine and triiodothyronine, by the thyroid gland by binding to receptors located on cells in the thyroid gland. Thyroxine and triiodothyronine are essential to maintaining the body's metabolic rate, heart and digestive functions, muscle control, brain development and maintenance of bones.

### Intended Use:

The TSH (Thyroid Stimulating Hormone) GENLISA™ ELISA is intended for the quantitative determination of TSH (Thyroid Stimulating Hormone) in human serum and plasma.

### Principle:

The TSH (Thyroid Stimulating Hormone) GENLISA™ ELISA method employs sandwich enzyme linked immunosorbent assay (ELISA) technique. Monoclonal anti-TSH are pre-coated onto microwells. Samples/Standards are pipetted into microwells and TSH present in the sample are bound by the antibodies. Monoclonal anti-TSH antibody conjugated to HRP conjugate is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (A and B) is added to microwells and color develops proportionally to the amount of TSH present in sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

### Materials Provided:

1. Microtiter Coated Plate (8x12 wells) - 1 no
2. TSH Standards (0.5 ml) - 0, 0.2, 1, 5, 10, 40 uIU/ml
3. Enzyme Conjugate - 6.5 ml
4. (40X) Wash Buffer - 20 ml
5. Sample Diluent - 11 ml
6. TMB Substrate - 12 ml
7. Stop Solution - 12 ml
8. Instruction Manual

### Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

### Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

### Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.



### Specimen Collection and Handling:

**Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

**Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

### Reagent Preparation:

1. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 2.5 ml of Wash Buffer (40X) to 97.5 ml of DI water. This is the working solution.
2. Allow all components to reach RT (Room Temperature) prior to use in the assay.

### Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **50 ul Samples and 50 ul of Standards** to the respective wells.
3. Add **50 ul Assay Diluent** to all the wells. Mix gently.
4. Dispense **50 ul Enzyme Conjugate** to all wells.
5. Shake gently for 30 seconds to mix well. Incubate at 37°C for 60 minutes.
6. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7. Add **100 ul of TMB Substrate** to all wells.
8. Incubate at 37°C for 10 minutes.
9. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

### Interpretation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown TSH concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the TSH Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit, 4-PL or a polynomial curve (2nd order) is best recommended for automated results.

**Note:**

It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 40 uIU/ml standard

**Positive Results:**

If the determination value is higher or lower than normal range, it means there is an abnormal result. The final result should be diagnosed in correlation with the clinical symptoms and other diagnostic methods.

**Criteria of Validation:**

TSH results are considered to be valid, if  
Correlation coefficient of the Standard Curve  $\geq 0.90$

*OD = Optical Density / Absorbance at 450nm*

**Reference Values:**

TSH normal concentration should be 0.26 - 4.0 uIU/ml.

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

**Limitations of Method:**

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

**Performance Characteristics:**

**Sensitivity:**

Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus  $2*SD$ .

10 replicates of '0' standards were evaluated and the LOD was found to be less than 0.05 uIU/ml

**Specificity:**

The monoclonal antibodies used in the kit are specific for TSH.

**Precision:**

Intra-Assay: CV%  $\leq 15\%$ .

Inter-Assay: CV%  $\leq 20\%$

**Safety Precautions:**

- **This kit is For In-vitro Diagnostic Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide ( $< 0.1\%$  w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.





- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

## LIMITED WARRANTY

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### Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

### SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add <b>50 ul Samples and 50 ul of Standards</b> to the respective wells.
3	Add <b>50 ul Assay Diluent</b> to all the wells. Mix gently.
4	Dispense <b>50 ul Enzyme Conjugate</b> to all the wells.
5	Shake gently for 30 seconds to mix well. <b>Incubate at 37°C for 60 minutes.</b>
6	Aspirate and <b>wash plate 5 times</b> with <b>(1X) Wash Buffer</b> and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7	Add <b>100 ul of TMB Substrate A</b> and <b>50 ul of TMB Substrate B</b> to all wells.
8	<b>Incubate at 37°C for 10 minutes.</b>
9	Add <b>100 ul of Stop Solution</b> . <b>Read result with an ELISA reader at 450 nm</b> within 15 minutes of stopping the reaction.

**SYMBOLS KEY**

	Microtiter Plate (8x12 wells)
	TSH Standards
	Enzyme Conjugate
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature