T4 (Thyroxine) GENLISA™ ELISA

REF : KBD402

Ver 1.0

IVD

Enzyme Immunoassay for the Quantitative Determination of T4 (Thyroxine) in human serum and plasma.

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

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REF KBD402 Σ 96 tests

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Introduction:

Thyroxine, also called 3,5,3′,5′-tetraiodothyronine, or T_4, one of the two major hormones secreted by the thyroid gland (the other is triiodothyronine). Thyroxine's principal function is to stimulate the consumption of oxygen and thus the metabolism of all cells and tissues in the body. Thyroxine is formed by the molecular addition of iodine to the amino acid tyrosine while the latter is bound to the protein thyroglobulin. Excessive secretion of thyroxine in the body is known as Hyperthyroidism and the deficient secretion of it is called Hypothyroidism.

Intended Use:

The T4 (Thyroxine) GENLISA™ ELISA is intended for the quantitative determination of T4 (Thyroxine) in human serum and plasma.

Principle:

The T4 (Thyroxine) GENLISA™ ELISA method is a quantitative determination based on competitive enzyme-linked immunosorbent assay (ELISA) to determine the level of T4 (Thyroxine) molecules in samples. Standards and Samples are added to the microtiter well which is pre-coated with purified anti-T4. T4 antigen conjugated to HRP is added to the microplate to compete with the T4 (Thyroxine) molecules present in the samples to form a complex. After incubation and a washing step, Substrate A and B, are added. Blue color develops on incubation and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the T4 (Thyroxine) molecules in the samples is inversely proportional to the yellow color developed (absorbance) in the wells.

Materials Provided:

1. Microtiter Coated Plate (8x12 wells) - 1 no
2. Standards (0.5 ml) - 0, 20, 40, 80, 160, 320 ng/ml
3. Assay Diluent - 6.5 ml
4. Enzyme Conjugate - 6.5 ml
5. (40X) Wash Buffer - 20 ml
6. Sample Diluent - 11 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. 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 37ºC 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 37ºC 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 T4 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 T4 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 320 ng/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:

T4 results are considered to be valid, if
Correlation coefficient of the Standard Curve >= 0.90

OD = Optical Density / Absorbance at 450nm

Reference Values:

T4 normal concentration should be 50 - 140 ng/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 5.0 ng/ml

Specificity:
The monoclonal antibodies used in the kit are specific for T4.

Precision:
Intra-Assay: CV% ≤15%.
Inter-Assay: CV% ≤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.

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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.
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4	Dispense **50 ul Enzyme Conjugate** to all the wells.
5	Shake gently for 30 seconds to mix well. **Incubate** at **37ºC** 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 **37ºC** 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.

SYMBOLS KEY

MTP	**Microtiter Plate (8x12 wells)**
STD	**T4 Standards**
ASSY DIL	**Assay Diluent**
CONJ	**Enzyme Conjugate**
SUB TMB A	**TMB Substrate A**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**