Testosterone Free GENLISA™ ELISA

REF : KBD379

Ver 2.1

IVD

Enzyme Immunoassay for Quantitative determination of Testosterone Free in 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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CE

REF KBD379 **Σ 96 tests**

Krishgen Pudgala LLP Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400018. India.
Tel: +91-22-49198700 | email: sales@krishgenpudgala.com

EC REP **KinesisDx, Lyoner Strasse 14, Frankfurt, Germany**

Introduction:

Testosterone is a steroid hormone belonging to an androgen group. It is the principal male sex hormone and the "original" anabolic steroid. It is derived from cholesterol. The largest amounts of testosterone are synthesized by testes, but is also synthesized in smaller quantities by the theca cells of the ovaries, the zona reticulosa of the adrenal cortex, and the placenta. Substantial amounts of the testosterone in women are also produced from estradiol by reverse aromatization in the liver, adipose cells, and other peripheral tissues. The mechanism includes: by activation of the androgen receptor (directly or as DHT), and by conversion to estradiol and activation of certain estrogen receptors. Testosterone Free is transported into the cytoplasm of target tissue cells, where it can bind to the androgen receptor, or can be reduced to dihydrotestosterone (DHT) by the cytoplasmic enzyme. It plays a major role in maintaining the secondary sexual characteristics in males, which are considered as virilizing effect; whereas anabolic effects include growth of muscle mass and strength, increased bone density and strength, etc.

Intended Use:

The Free Testosterone GENLISA™ ELISA is intended for the quantitative determination of Free Testosterone in human serum and plasma.

Principle:

Testosterone Free GENLISA™ ELISA method is based on a competitive enzyme-linked immunosorbent assay (ELISA) to determine the level of free Testosterone present in serum and plasma. Standards, Samples, Controls are added to the microtiter well which is pre-coated with anti-Testosterone Free antibody. Enzyme conjugate antigen is added to the microplate to compete with the Testosterone Free present in the samples to form a complex. After incubation and a washing step, substrate solution is added. Blue color develops on incubation and the reaction is stopped with a stop solution to develop a yellow color. The concentration of Testosterone Free in the samples is inversely proportional to the yellow color developed (absorbance) in the wells.

Materials Provided:

1. Coated Microtiter Plate (96 wells) – 1 no
2. Standards (1 ml/vial) – 0, 0.2, 1.0, 4.0, 20.0, 100.0 pg/ml
3. High Control – 1 ml
4. Low Control – 1 ml
5. Enzyme Conjugate – 15 ml
6. (20X) Wash Buffer – 2 x 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution –12 ml
9. Instruction Manual – 1 no

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 minutes at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.

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

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. (1X) Wash Buffer Dilution: To make (1X) Wash Buffer, add 25ml of (20X) Wash Buffer to 475ml of DI water. This is the working solution.

Test Procedure:

1. Add **20 ul Sample, Standards, Controls** into appropriate wells.

2. Add **100 ul Enzyme Conjugate** into each well. Mix well.

3. Incubate at 37°C for **60 minutes**.

3. 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.

4. Add **100 ul** of **TMB Substrate** to all wells.

5. Incubate at RT for 15 minutes.

6. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 5 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate 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 Testosterone Free 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 Testosterone Free Concentration. If samples were diluted, multiply by the appropriate dilution factor.

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

To convert results to mass units:
pg/mL = pmol/L x 0.289

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 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.

Control Range:

Low Control: 0.78 – 1.70 pg/ml
High Control: 9.09 – 18.49 pg/ml

Reference Values:

	No. of Subjects	Median pg/ml	Reference Interval (pg/ml)
Males			
21 – 49 years	120	14.13	5.01 – 27.78
> 50 years	120	12.75	4.11 – 21.85
Females			
Pre - Menopausal	120	0.55	< LOQ – 1.70
Post - Menopausal	120	0.75	< LOQ – 2.34

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.

Precision:
Intra-Assay: <11.5 %CV
Inter-Assay: <15% CV

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.

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

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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Krishgen Pudgala LLP Unit No.1/2, Om Sainath Commercial Complex,
Off Mankoli-Anjur Phata Road. Village Dapode, Bhiwandi 421302.

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 **20 ul Sample, Standards, Controls** into appropriate wells.
3	Add **100 ul Conjugate Working Solution** into each well. Mix well. Incubate at **37°C** for **60 minutes.**
4	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.
5	Add **100 ul** of **TMB Substrate** to all wells.
6	Incubate on shaker at RT for **10 minutes.**
7	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 (8 x 12 wells)**
HIGH CONTRL	**High Control**
LOW CONTRL	**Low Control**
STD	**Standards**
ENZY CONJ	**Enzyme Conjugate**
20x WASH BUF	**(20x) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**