FSH GENLISA™ ELISA

REF : KBD381

Ver 2.0

IVD

Enzyme Immunoassay for Quantitative Determination of FSH 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**

REF KBD381 Σ 96 tests

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Introduction:

Follicle stimulating hormone is one of the gonadotrophic hormones, secreted by pituitary gland into the bloodstream. Follicle stimulating hormone is one of the hormones essential to pubertal development and the function of women's ovaries and men's testes. In women, this hormone stimulates the growth of ovarian follicles in the ovary before the release of an egg from one follicle at ovulation. It also increases estradiol production. In men, follicle stimulating hormone acts on the Sertoli cells of the testes to stimulate sperm production (spermatogenesis).

Intended Use:

The FSH GENLISA™ ELISA is intended for the quantitative determination of Follicle Stimulating Hormone in serum and plasma.

Principle:

The FSH GENLISA™ ELISA method employs sandwich enzyme linked immunosorbent assay (ELISA) technique. Monoclonal Anti-FSH Antibodies are pre-coated onto microwells. Standards/Samples/Controls are pipetted into microwells and Follicle Stimulating Hormone present in the sample is bound by the antibodies. Enzyme conjugate antibody is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the ready to use TMB substrate is added to microwells and color develops proportionally to the amount of Follicle Stimulating Hormone 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. Standard 0 (2 ml/vial) - 0 mIU/ml
3. Standards (0.5 ml/vial) - 5, 10, 20, 50, 100 mIU/ml
4. Control Serum - 0.5 ml
5. Enzyme Conjugate - 12 ml
6. (25X) Wash Buffer – 2 X 25 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 4 ml of Wash Buffer (25X) to 96 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 **25 ul Samples, Standard, Control Serum** into appropriate wells.
3. Add **100 ul Enzyme Conjugate** in each well.
4. Incubate at room temperature for 90 minutes.
5. 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.
6. Add **100 ul** of **TMB Substrate** to all wells.
7. Incubate at room temperature for 10-15 minutes.
8. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Calculations:

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 FSH 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 FSH 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 sample reads more than 100 mIU/ml, then dilute with Standard 0. The obtained result should be multiplied by the dilution factor.

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.

Reference Value:

Group	Phase	Range (mIU/ml)
Males	-	1.4-14
Females	Follicular Stage	< 10
	Midcycle Peak	5-16
	Luteal Phase	< 10
	Postmenopausal	25-150

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. 12 replicates of '0' standards were evaluated and the LOD was found to be 0.3 mIU/ml.

Specificity:

Cross-reagent	Cross reactivity, %
FSH	100
hCG	0.001
TSH	0.0000
LH	0.019

Precision:

Intra-Assay-Variation

Sample	N	Mean Value	Standard Deviation	CV (%)
1	9	10.1	0.450	4.5

Inter-Assay-Variation

Sample	Mean Value	Standard Deviation	CV (%)
1	9.7	0.442	4.6

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 **25 ul Sample, Standard, Control Serum** into appropriate wells.
3	Add **100 ul Enzyme Conjugate** in each well. Incubate at room temperature for 90 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.
7	Add **100 ul** of **TMB Substrate** to all wells.
8	Incubate at **room temperature** for **10-15 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)**
CTRL	**Control Serum**
STD	**Standards**
ENZY CONJ	**Enzyme Conjugate**
25X WASH BUF	**(25X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**