Anti Mullerian Hormone (AMH) GENLISA™ ELISA

REF : KBD601

Ver 1.2

IVD

Enzyme Immunoassay for the Qualitative Determination of Anti-Mullerian Hormone (AMH) antibody 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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CE REF KBD601 Σ 96 tests

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Introduction:

Anti-Mullerian hormone (AMH), a peptide growth factor of the transforming growth factor-β family, is well known for its role in sexual differentiation. In men, AMH is secreted from the Sertoli cells of the testes, promotes Mullerian duct regression, and initiates male phenotypic development. In women anti-Mullerian hormone (AMH) is produced in the ovary by granulosa cells of antral follicles. The hormone plays a significant role in the development of reproductive organs in both sexes during the embryonic period. A gradual increase in AMH levels is observed in girls from the first day of life, with maximum levels observed in women at around the age of 25. In an adult woman AMH levels gradually decrease until they reach values below detectable limits in postmenopausal women.

Intended Use:

The Anti Mullerian Hormone (AMH) GENLISA™ ELISA is intended for the quantitative determination of Anti Mullerian Hormone (AMH) in human serum and plasma.

Principle:

The Anti Mullerian Hormone (AMH) GENLISA™ ELISA method employs sandwich enzyme linked immunosorbent assay (ELISA) technique. Monoclonal antibodies are pre-coated onto microwells. Samples and Standards are pipetted into microwells and AMH present in the sample are bound by the antibodies. Biotinylated AMH antibody is added which binds to the bound antibody-antigen complex. This is followed by addition of Streptavidin-HRP which binds to antibody-antigen - biotin complex. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution is added to microwells and color develops proportionally to the amount of AMH 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 (96 wells) – 1 no
2. Lyophilized Standard (1ug/ml concentration) – 2 vials
3. Biotinylated AMH Antibody – 6 ml
4. Streptavidin HRP Conjugate Concentrated – 100 ul
5. Sample Diluent – 25 ml
6. (20x) Wash Buffer – 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 5 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, recentrifuge.

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, recentrifuge.
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Reagent Preparation:

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 25 ml of 20X Wash Buffer in 475 ml of DI water. This is the working solution.
4. To make Streptavidin HRP (1X); dilute 5.4 ul of Streptavidin HRP concentrated in 2 ml of Sample Diluent. Mix well before use. Streptavidin HRP is recommended to be prepared fresh before use.
5. Standards Preparation: Reconstitute the lyophilized standard with 1 ml of Sample Diluent. Keep the reconstituted standard for 15 mins with gentle agitation.
   Dilute 4 ul of original Standard (1 ug/ml) with 196 ul of Sample diluent to generate a 20 ng/ml Standard stock solution. Keep the standard for 15 mins with gentle agitation before making further dilutions. Prepare the Standards by serially diluting the standard stock solution as per the below table. The Sample Diluent acts as the '0' standard.

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Standard Concentration	Standard Vial	Dilution Particulars
1 ug/ml	Original Standard	Original Standard provided in the Kit
20 ng/ml	Standard No.5	4 ul Standard Provided (40 ng/ml) + 196 ul Standard Diluent
10 ng/ml	Standard No.4	100 ul Standard No.5 + 100 ul Standard Diluent
5 ng/ml	Standard No.3	100 ul Standard No.4 + 100 ul Standard Diluent
2.5 ng/ml	Standard No.2	100 ul Standard No.3 + 100 ul Standard Diluent
1.25 ng/ml	Standard No.1	100 ul Standard No.2 + 100 ul Standard Diluent
0 ng/ml	Standard No. 0	100 ul of Sample Diluent

Note: It is recommended to use reconstituted standards within two hours after reconstitution.

Assay Procedure:

1. All reagents should be allowed to reach room temperature before use.

2. Add 25 ul Sample, Standards into appropriate wells.

3. Add 50 ul biotinylated AMH Antibody to each 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 diluted Streptavidin HRP Conjugate into each well. Mix well. Incubate at 37°C for 30 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 15 minutes.

9. Add 100 ul of Stop Solution.

10. Read result with an ELISA reader at 450 nm within 10 minutes of stopping the reaction.

Calculation 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 AMH 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 AMH 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 with concentrations greater than the highest standard have to be further diluted or reported as > 20 ng/ml. For calculation of concentrations, consider this 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 Range:

Sample	AMH Range (ng/mL)
Females < 8 wks.	<0.02 – 0.49
Females < 10 yrs.	0.05-10.40
Females 11-20 yrs.	0.62 – 11.00
Females 21-30 yrs.	<0.02 – 10.39
Females 31-40 yrs.	0.14 – 10.40
Females 41-50 yrs.	<0.02 – 6.35
Females > 51 yrs.	<0.02- 0.39
Males < 3 days	25.9 – 69.1
Males < 3 Month.	24.22 – 275.46
Males 1-11 yrs.	38.25 – 332.40
Males 12-20 yrs.	1.12 – 143.64
Males 20 yrs.	0.59 – 17.71

Note: It is recommended that each laboratory should determine the reference range for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information**.**

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 < 0.5 ng/ml

Specificity:
The antibodies used in the kit are monoclonal antibodies, specific for AMH. The calibrators / standards used are calibrated against Anti-Mullerian Hormone.

Precision:
Inter-Assay: ≤15%
Intra-Assay: ≤10%

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.
2	Add **25 ul Sample, Standards** into appropriate wells.
3	Add **50 ul biotinylated AMH Antibody** to each 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 Diluted Streptavidin HRP Conjugate** into each well. Mix well. Incubate at **37°C** for **30 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.

SYMBOLS KEY

MTP	**Microtiter Plate (8x12 wells)**
STD	**Standards**
BIO Ab	**Biotinylated AMH ANTIBODY**
HRP CONJ	**Streptavidin HRP Conjugate**
SAMP DIL	**Sample Diluent**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
STOP SOLN	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**