Pariksha Biotech
A game changer in IVD

AMH Estimation Kit

High-Q Anti-Mullerian Hormone

Latex Enhanced Turbidimetric Immuno Assay (LETIA)

HIGH Q
Command on Quality

Summary :
The anti-Müllerian hormone is a homodimeric glycoprotein belonging to the transforming growth factor β (TGF β) family. All members of this superfamily are involved in the regulation of tissue growth and differentiation. Prior to secretion, the hormone undergoes glycosylation and dimerization to produce an approximately 140 kDa precursor of two identical disulfide linked 70 kDa subunits. Each monomer contains a large N-terminal pro region and a much smaller C-terminal mature domain. In contrast to other TGF β family members, AMH is thought to require the N-terminal domain topotentiate activity of the C-terminal domain to attain full bioactivity. A part of AMH is then cleaved at a specific site between the pro-region and the mature region during cytoplasmic transit to generate biologically active110 kDa N-terminal and 25 kDa C-terminal homodimers which remain associated in a non-covalent complex. The AMH type II receptor (AMH RII) has the capacity of binding only the biologically active form of AMH. In males, AMH is secreted by the Sertoli cells of the testes. During embryonic development in males, secretion of AMH from testicular Sertoli cells is responsible for the regression of the Müllerian duct and the normal development of the male reproductive tract. The secretion of AMH by the Sertoli cells starts during the embryogenesis and continues throughout life. AMH is continuously produced by the testicles until puberty and then decreases slowly to post-puberty values. In females AMH plays an important role in the ovarian folliculogenesis. Follicle development in the ovaries comprises two distinct stages: Initial recruitment, by which primordial follicles start to mature, and Cyclic recruitment, which leads to the growth of a cohort of small antral follicles, among which the dominant follicle (destined to ovulate) is subsequently selected. FSH directs the cyclic recruitment. AMH expression in granulosa cells starts in primary follicles and is maximal in granulosa cells of pre antral and small antral follicles up to approximately 6 mm in diameter. When follicle growth becomes FSH-dependent, AMH expression diminishes and becomes undetectable. This pattern of AMH expression supports the inhibitory role of AMH at two distinct stages of folliculogenesis. First, AMH inhibits the transition of follicles from primordial into maturation stages and thereby has an important role in regulating the number of follicles remaining in the primordial pool. Second, AMH has inhibitory effects on follicular sensitivity to FSH and therefore has a role in the process of follicular selection. Serum levels of AMH are barely detectable at birth in females, reach their highest levels after puberty, decrease progressively thereafter with age, and become undetectable at menopause. Serum AMH levels have been shown to be relatively stable during the menstrual cycle with substantial fluctuations being observed in younger women. AMH levels further demonstrate lower intra- and inter-cyclic variation than baseline FSH. Serum AMH levels decrease significantly during the use of combined contraceptives.Clinical applications of AMH measurements have been proposed for a variety of indications. Measurement of serum AMH is clinically mainly used for assessment of ovarian reserve reflecting the number of antral and pre-antral follicles, the so-called Antral Follicle Count (AFC), and for the prediction of response to controlled ovarian stimulation. Further clinical applications of AMH are diagnosis of disorders of sex development in children and monitoring of granulosa cell tumors to detect residual or recurrent disease. AMH has been suggested as a surrogate biomarker for AFC in the diagnosis of Polycystic Ovary Syndrome (PCOS) and for the prediction of time to menopause.

Principle:
Latex particles coated with antibody specific to human AMH aggregate in the presence of AMH from the sample forming immune complexes. The immune complexes result in turbidity of the reaction solution. The turbidity is proportional to the content of the AMH antigen in the sample. The AMH concentration is determined from a calibration curve developed from AMH calibrators of known concentration.

Instrument
The reagents can be used on most Semi auto and automated chemistry analyzers.

Specimen Collection and Storage
1. Fresh serum without hemolysis is the specimen of choice.
2. Specimens can be stored at 2 - 8 °C for 24 hours. If the specimens have to stored for more than 24 hours, it's suggested that the specimens have to be frozen below -20 °C. Avoid repeated freeze-thaw cycles.
3. Highly turbid and hemolyzed samples must not be tested for AMH

Reagents, Calibartors and Controls storage and Shelf life:
The High -Q AMH reagents, Calibrators and Controls should be stored at 2-8°C and are are stable till the expiry date mentioned on the labels . Do not freeze the reagents

High-Q AMH Calibrators are provided as 4 Levels of Liquid Calibrators and are ready to use. Calibrators are stable up to the expiry date mentioned on the labels. Aliquot it in to small volumes and store at 2-8°C for the contamination free use. Do not freeze the Calibrators

High-Q AMH Controls are provided optionally as 2 Levels of Liquid Controls. Controls are stable up to the expiry date mentioned on the labels. Aliquot it in to small volumes and store at 2-8°C for the contamination free use. Do not freeze the Controls

Reagents

Reagents	Appearance
Reagent 1 (R1)	Colourless Liquid
Reagent 2 (R2)	Turbilatex
AMH Calibrator	Level 1-4 Liquid Stable Calibrators
AMH Control	Liquid Stable Controls

Kit Components and Composition:

Name	Description	Con. Value
Reagent 1 (R1)	Neutral-weakly basic buffer	0.05-0.5 mol/L
Reagent 2 (R2)	Neutral-weakly basic buffer	0.05-0.5 mol/L
	Anti-AMH antibody immobilized latex beads	0.05-0.3 %
AMH Calibrator	Recombinant AMH antigen	Lot Specific
	Excipient	-
	Neutral-weakly basic buffer	0.05-0.5 mol/L
AMH Control	Recombinant AMH antigen	Lot Specific
	Excipient	-

Performance Characterestics:
1. Linearity: In the range of [0.0 - 24.0] ng/mL, the correlation coefficient of linear regression should not be less than 0.990.
2. Repeatability: When the quality controls are repeatedly measured in the same batch of the kits, the coefficient of variation (CV) of the results should not exceed 10%.
3. Validity of quality control's value assignment: The test results of the quality controls are within the target range.
4. Analytical sensitivity:

LoB	<1.0ng/mL
LoD	≤ 0.01ng/mL
LoQ	≤ 24.0ng/mL

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Assay Procedure: (Multi Point Calibration with 4 Level Calibrators)

System Parameters:

Reaction Type (Mode)	**Fixed Time-Multipoint Linear/Point to Point**
Reaction Direction	Increasing
Delay Time	30 Seconds
Measuring Time	300 Seconds
Wave Length	630nm (600-670 nms)
Flow Cell Temp.	37°C
Reagent Volume	375 µl (R1) + 125 µl (R2)
Sample Volume)	100 µl
Calibrator Concentration	(On the Vials Lot Specific)
Linearity	24 ng/mL

Reagent	Calibrator	Sample/Control
AMH R1	375 µl	375 µl
Calibrator	100 µl	----
Serum Sample	----	100 µl
Mix and incubate for 5 Minutes at 37 °C		
AMH R2	125 µl	125 µl

Read absorbance (A) at 630 nms (600-670 nms) for all the Calibrators/ Controls and Samples

Calculations with Calibrators/ Calibration Curve/ Result Interpretation:
Calculate the Δ Absorbance of Calibrators = Abs of Calibrator
Plot the Δ absorbances of all the Calibrators versus their respective concentrations on a linear graph paper. AMH Results for the samples and controls are determined using the prepared calibration curve.
Δ Abs of Sample ie Abs of Sample
AMH in the sample is calculated by interpolation of Abs of Sample on the calibration curve.

Calculation

The concentration of AMH in unknown samples is derived from a calibration curve using an appropriate mathematical model such as Multi Point Linear or Point to Point. The calibration curve is obtained with 4 calibrators at different levels. Stability of calibration: 4 weeks

Reference Values:

Adult Females

18 - 25 yrs : 0.96 -13.34 ng/ml
26 - 30 yrs : 0.49 - 8.50 ng/ml
31 - 35 yrs : 0.25 - 7.35 ng/ml
36 - 40 yrs : 0.15- 7.15 ng/ml
41 - 45 yrs : 0.01 - 3.27 ng/ml
45 - 50 yrs : 0.00 - 1.15 ng/ml

High AMH Levels are predictive of Ovarian Hyperstimulation Syndrome/PCOs. Polycystic ovarian syndrome can elevate AMH higher than age-specific reference ranges and predict anovulatory, irregular cycles. Ovarian tumours like Granulosa cell tumour are often associated with higher AMH.

The above reference ranges are given as a guide. It is recommended that each laboratory should establish its own reference values from a rigorously selected population. All the results should be clinically correlated

Explanation:

The test results shall be influenced by many factors (patients' age, gender, geographical regions, etc. Basically, the results are considered as normal if they fall within the reference range. To the contrary, they should be redetermined for confirmation. If they are obviously beyond the reference range the reference range after confirmatory tests, it is considered that the AMH in serum is abnormal. If the test results are inconsistent with the clinical results, the reasons should be analyzed and found out.

Interpretation:

IN MALES- it is used to evaluate testicular presence and function in infants with intersex conditions or ambiguous genitalia, and to distinguish between cryptorchidism and anorchia in males.

IN FEMALES-During reproductive age, follicular AMH production begins during the primary stage, peaks in preantral stage & has influence on follicular sensitivity to FSH which is important in selection for follicular dominance. AMH levels thus represent the pool or number of primordial follicles but not the quality of oocytes. AMH does not vary significantly during menstrual cycle & hence can be measured independently of day of cycle.

• Obese women are often associated with diminished ovarian reserve & can have 65% lower mean AMH levels than non obese women.

• A combination of Age, Ultrasound markers -ovarian volume and Antral follicle count, AMH level & FSH level are useful for optimal assessment of ovarian reserve. Studies in various fertility clinics are ongoing to establish optimal AMH concentrations for predicting response to Invitro Fertilization

• AMH decreases with concurrent increase in Testosterone during puberty

References;

1. Guidelines for the preparation of in vitro diagnostic reagents instructions, State Food and Drug Administration, 2014.
2. Yue C-Y, Lu L-k-y, Li M, Zhang Q-L, YingC-M (2018) Threshold value of anti-Mullerian hormone for the diagnosis of polycystic ovary syndrome in Chinese women. PLoS ONE 13(8): e0203129

Ordering Information

Ref./Cat. No.	Pack Size	Presentation
P - AMH-25	25 ml	Two Liquid Reagents with Calibrator Set

Product Features

- **Two liquid reagents**
- **4 Level Liquid Calibrator set provided**
- **Linearity : 0.0 - 24.0 ng/mL**
- **Multipoint Linear Assay (Point to Point Assay)**
- **10 Minutes Assay (5 Minutes First Incubation (30 Sec Delay and 300 Sec Measuring)**
- **Can be used on semi and fully auto analyzers**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic medical device		Keep away from sunlight
	Do not freeze		This way up
	Use-by date		Do not use if package is damaged
	Keep dry	LOT	Batch Code
	Consult instructions for use	R	Reagent
eIFU Indicator	Consult electronic instructions for use	CAL	Calibrator Material
REF	Catalogue number		
	Caution		

eIFU Indicator

Pariksha's world inside
SCAN TO EXPLORE MORE

Manufactured in India by :
Pariksha Biotech Pvt Ltd,
Plot no.1/B-14, SVICE,
Balanagar,
Hyderabad-500037
Telangana State

+91 7075706709
info@parikshabio.com
www.parikshabio.com