TRUSTwell®

Anti-Mullerian Hormone (AMH) ELISA Kit

REF

AE1240

IVD

- 48-well ELISA kit for the quantitative determination of the concentration of Anti-Mullerian hormone (AMH) in human serum or plasma
- Store at 2-8°C upon receipt

INTENDED USE

The TRUSTwell Anti-Mullerian hormone (AMH) ELISA is a solid-phase enzyme-linked immunosorbent assay for the quantitative determination of AMH concentration in human serum or plasma. It is intended to be used by trained professionals as an aid in assessing woman's ovarian reserve.

INTRODUCTION

Anti-Müllerian hormone (AMH) or Müllerian inhibiting substance (MIS) is a glycoprotein consisting of two 70-kDa subunits and a member of the transforming growth factor-β super family. In females, AMH is at detectable range throughout the reproductive period, slowly diminishes during the reproductive senescence and it becomes undetectable during post-menopause. Measurement of AMH has been observed as a reliable assessment of the ovarian reserve[1]. Unlike FSH, the variability of the AMH measurement within the mensural cycle is small, hence it has clinical significance in determining ovarian reserve especially in sub-fertile women prior to ovulation induction, estimation of time of menopause[2], diagnosis and follow-up of polycystic ovary syndrome (PCOS)[3,4]. Women with PCOS show 2 or 3 fold higher serum AMH level than normally ovulating women[5]. The AMH levels have also been used to evaluate infants with ambiguous genitalia[6]. Recently, AMH levels have become a primary marker for female fertility and an important indicator in the treatment and management in female infertility. A fertile woman has AMH levels ranging from 1.0-4.0 ng/mL, while a woman with an AMH level <1.0 ng/mL is considered to have a low ovarian reserve.

TEST PRINCIPLE

The TRUSTwell AMH ELISA is a quantitative solid-phase enzyme-linked immunosorbent assay, based on the principle of antibody sandwich technique, for the measurement of AMH concentration in human serum or plasma.

The TRUSTwell AMH ELISA is comprised of three key components:

1) Solid microwells pre-coated with AMH antibody.
2) AMH calibrators and controls.
3) AMH Biotin-Ab reagent and AMH enzyme reagent comprised of horse radish peroxidase conjugated to streptavidin.

During the assay, the calibrator, control, or test specimen as well as the biotin reagent containing the Anti-AMH-Ab-Biotin is added to the AMH-antibody coated microwell. During the 60-minute incubation, the AMH in the test specimen binds to both the coated antibody and the Biotin-Ab forming the sandwich-complex.

Unbound material is then removed by washing. In the next step, HRP conjugated streptavidin is added, then washed after incubation. Next, TMB single Substrate is added and the presence of the HRP bound to microwell surface is shown by the development of a blue color. The reaction is then terminated with the Stop Solution and the optical density (OD) is determined using a spectrophotometer at 450/620-690 nm. The color intensity reflects the amount of Ab-HRP bound to the AMH-Ab coated microwell, and is directly proportional to the amount of AMH in the control or test specimen.

A dose-response curve can then be developed by plotting the AMH calibrator concentrations on the x-axis against the relative OD values on the y-axis. The AMH concentration of each assay control or test specimen can then be interpolated from the dose-response curve.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	AMH Antibody Coated Microwells	8 wells x 6 strips	AE1240W
2	AMH Calibrators*: C1 (0.0 ng/mL)	0.25 mL	AE1240C1
3	C2 (0.2 ng/mL)	0.25 mL	AE1240C2
4	C3 (0.5 ng/mL)	0.25 mL	AE1240C3
5	C4 (1.0 ng/mL)	0.25 mL	AE1240C4
6	C5 (5.0 ng/mL)	0.25 mL	AE1240C5
7	C6 (15.0 ng/mL)	0.25 mL	AE1240C6
8	AMH Assay Control A	0.25 mL	AE1240ACA
9	AMH Assay Control B	0.25 mL	AE1240ACB
10	AMH Biotin Ab (51X)	0.25 mL	AE1240B
11	AMH Biotin Ab Diluent	12 mL	AE1240BD
12	AMH Enzyme Reagent	12 mL	AE1240H
13	Wash Buffer Concentrate (30X)	15 mL	AWE3000
14	TMB Substrate	7 mL	ATME2000
15	Stop Solution	6 mL	ASE1000
16	Instructions for Use	1	PI-AE1240
17	Working Sheet	2 No's	AE0001ES
18	Sealent	2 No's	
19	Desiccant	4 No's	

*The Assay Control and calibrators values are lot-specific. Please see vial labels for exact concentration ranges.

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 50 µL, 100 µL, and 1 mL
2. Microplate reader
3. Microplate washer
4. Vortex mixer or equivalent
5. Absorbent paper for blotting the microwells
6. Graph paper
7. Timer
8. Distilled or de-ionized water

STORAGE AND STABILITY

All reagents except the concentrated wash buffer, calibrators and controls are ready for use as supplied. Store all components at 2-8°C. Ensure that the reagents are brought to room temperature before opening. After removing the desired number of wells, place unused wells in the resealable foil pouch provided with desiccant and return to 2-8°C.

SPECIMEN COLLECTION AND PREPARATION

- Whole blood should be obtained in a redtop venipuncture tube without additives or anti-coagulants to prepare serum specimens, or in EDTA/heparin-containing tubes to prepare plasma specimens. Fasting morning serum sample should be obtained to achieve accurate comparison to the established normal values.
- If not tested immediately, the specimens can be stored at 2-8°C for up to 7 days,or frozen at -20°C for up to 30 days. Avoid multiple freeze-thaw cycles. If a specimen is to be shipped, pack in compliance with federal regulations covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation before testing.
- Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity. Specimens containing sodium azide may interfere with test results.

1. Bring all reagents to room temperature (22-28°C).
2. **Preparation of working Wash Buffer:**
   48 wells – 10 mL of 30 X wash buffer in 290 mL of DI water
3. Preparation of AMH Biotin antibody:

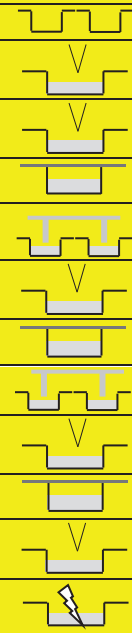
No.of Strips	1	2	3	4	5	6
AMH Biotin Ab (51 X) uL	20	40	60	80	100	120
Biotin Diluent (mL)	1.0	2.0	3.0	4.0	5.0	6.0

4. Determine the number of strips needed. Calibrators should be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Calculate the desired number of microwells. Remove the remaining microwells and place them with desiccant into the resealable foil pouch, seal and store at 2-8°C for later use.
2. Add 25 µL of AMH calibrators & AMH Assay Controls and test specimens into the assigned wells, respectively.
3. Add 100 µL of AMH Biotin Reagent into all wells.
4. Cover the plate with a microplate sealer and mix gently for 10 seconds.
5. Incubate the microplate at room temperature (22-28°C) for 60 minutes.
6. Wash Step (can be performed manually or with automated washing):
   Manual washing: carefully remove the incubation mixture by disposing the solution into a waste container. Fill each well with 350 µL of working wash buffer and mix gently for 20-30 seconds. Discard the wash solution completely. Repeat 4 more times. After completing the last wash step, tap the plate on absorbent paper to remove residual liquid.
   Automated washing: automatic plate washer must be calibrated to ensure efficient washing. Fill each well with 350 µL of diluted wash buffer. Aspirate all wells completely. Repeat 4 more times.
7. Add 100 µL of Enzyme reagent into each well. Incubate the microplate at room temperature (22-28°C) for 30 minutes. Wash the plate as described at step 6.
8. Add 100 µL of TMB Substrate into each well.TMB substrate solution must be colorless when used; if the solution turns blue, it must be replaced
9. Cover the microplate and incubate at room temperature (22-28°C) for 15 minutes.
10. Stop the reaction by adding 50 µL of Stop Solution to each well. Gently mix for 10 seconds. Add the Stop Solution in the same sequence as substrate addition. It is important to make sure that all the blue color changes completely to a yellow color.
11. Set the microplate reader wavelength at 450 nm. Measure the OD value of each well within 10 minutes after adding the Stop Solution. A filter of 620-690 nm can be used as a reference wavelength to optimize the assay result.

Flow chart of assay procedure

		
1.	Secure strips in microwell frame	Number of strips
2.	Add AMH Calibrators, Assay Controls and Test specimens into assigned wells.	25 µL
3.	Add AMH Biotin Reagent and Gently rock	100 µL
4.	Incubate at RT (22-28°C)	60 minutes
5.	Wash: manual or automatic	5 times
6.	Add AMH Enzyme Conjugate	100 µL 20 seconds
7.	Incubate at RT (22-28°C)	30 minutes
8.	Wash: manual or automatic	5 times
9.	Add ready to use TMB substrate Gently rock	100 µL 20 seconds
10.	Incubate at RT (22-28°C)	15 minutes
11.	Add Stop Solution. Gently rock	50 µL 20 seconds
12.	Read the result	450/620-690 nm within 10 minutes

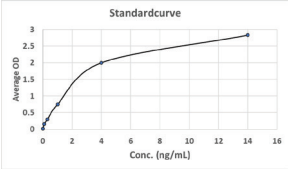
CALCULATION OF RESULTS

1. Calculate the mean OD value (A450/620-690) for each set of calibrators.
2. Construct a standard curve by plotting the mean OD value obtained for each calibrator against its concentration on graph paper, with OD values on the vertical y-axis, and concentrations on the horizontal x-axis.
3. Interpolate concentrations for the assay controls and test specimens by plotting their OD values on the curve.
   Alternatively, if software is used, calculate the concentration of AMH following the software menu.
4. If the OD value of a specimen is greater than that of the highest calibrator, it is recommended to dilute the specimen with C1 calibrator in a 1:1 or 1:3 ratio and test again. Any values obtained for a diluted sample must be further converted by applying the appropriate dilution factor in the calculation.

INTERPRETATION

Results of a typical standard curve are shown below:

Cal ID	Conc. (ng/mL)	OD
C1	0.0	0.013
C2	0.2	0.158
C3	0.5	0.293
C4	1.0	0.745
C5	5.0	2.000
C6	15.0	2.829
Patient ID	**Conc. (ng/mL)**	**OD**
1	1.584	0.989



The above data and figure are for example purposes only, and should not be used to calculate your result. Test was interpolated from the dose response curve (DRC)

QUALITY CONTROL

The following criteria should be met to consider assay results to be valid:

The mean OD of the '0' calibrator should be < 0.020.
CV's for duplicates of calibrators should be < 10%.
AMH Assay Control concentrations should be within the specified range on the vial labels, if not reject the test results

NORMAL REFERENCE

AMH concentration in female specimens from different age groups was measured with TRUSTwell AMH ELISA kits and the results presented below. It is recommended that each laboratory establishes its own normal ranges based on a representative sampling of the local patient population and its own assay technique. The following values for the TRUSTwell AMH ELISA can be used as initial guideline ranges only:

Normal values for AMH (ng/mL)

Age group	n	Mean	Range
20-29	32	6.10	1.77-12.42
30-39	93	2.72	0.11-12.67
40-49	42	1.31	0.02-9.76

The normal AMH concentration range for males and untested female age groups not covered in the above table is shown below, as obtained from published literature 7.

Male	Female
<24 months: 14-466 ng/mL	<24 months: <4.7 ng/mL
24 months-12 years: 7.4-243 ng/mL	24 months-12 years: <8.8 ng/mL
>12 years: 0.7-19 ng/mL	13-45 years: 0.9-9.5 ng/mL
	>45 years: <1.0 ng/mL

PERFORMANCE CHARACTERISTICS

1) Analytical Sensitivity

OD values from 20 replicates of the '0' calibrator was run in a single assay on three lots and the mean and standard deviation (SD) were calculated. The analytical sensitivity of the TRUSTwell AMH ELISA was determined to be 0.023 ng/mL at 2SD.

2) Specificity

The specificity of the TRUSTwell AMH ELISA was evaluated relative to the analytes shown on the table below. No cross-reactivity was observed at the concentrations listed

Analyte	Concentration	% Reactivity
Follicle Simulating Hormone	100 mIU/ml	<0.001
Human Chorionic Gonadotropin	1000 mIU/ml	<0.001
Luteinizing Hormone	200 mIU/ml	<0.001
Prolactin	100 ng/ml	<0.001

3) Precision

Intra-assay precision: Twenty replicates of each of three pooled human serum controls (low, medium, and high concentrations) were tested in the same assay. The mean, SD, and coefficient of variation (CV) were determined. The results are showed in the following table:

Sample	N	Mean (ng/mL)	SD	CV(%)
Low	20	0.86	0.16	0.35
Medium	20	4.78	0.86	0.06
High	20	13.15	0.33	2.53

Inter-Assay Precision: Three human serum pooled controls (low, middle, and high concentrations) were assayed in duplicates. The mean, SD, and CV were determined. The results are shown in the following table:

Sample	N	Mean (ng/mL)	SD	CV (%)
Low	16	0.86	0.02	1.9
Medium	16	4.8	0.01	0.5
High	16	12.64	0.43	3.46

WARNING AND PRECAUTIONS

For in Vitro Diagnostic Use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
2. Do not use expired test kits,
3. Bring all reagents to room temperature (22-28°C) before use.
4. Do not use a component from any other test kit as a substitute for the components in this kit. Do not re-use the used microwells to test new samples.
5. Do not use serum derived from hemolyzed blood specimens for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mouth. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform this test as bio-hazardous waste.
10. Prior to the first incubation, and after addition of the Stop Solution, gently shake the microwells to ensure thorough mixing. Avoid splashing liquid while shaking. Do not allow formation of air bubbles in the microwell.
11. Do not allow the microwells to dry between the end of the washing operation and the reagent distribution.
12. The enzyme substrate reaction is very sensitive to metal ions. Thus, do not llow any metal elements to come into contact with the conjugate or TMB Substrate.
13. The enzyme-substrate reaction is temperature dependent. Ensure that the room temperature is between 22-28˚C during the incubation.
14. Use a new dispensing tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate.
15. The wash procedure is critical. Wells must be aspirated completely before adding the Wash buffer or liquid reagents. Automatic washers must be validated with the test kit prior to use. Insufficient washing will result in poor precision and falsely elevated OD values.
16. Microplate reader must be calibrated per manufacturer's instruction to ensure accurate OD readings. A non-calibrated reader may lead to invalid test results.
17. Avoid exposure to strong light during color development.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Results must be followed closely when testing for AMH concentration in human specimens. Failure to follow the procedure may lead to inaccurate results.
2. To store the calibrators and controls for a longer period and multiple use, aliquot into cryo vials and store at -20°C. DO NOT FREEZE/THAW MORE THAN TWICE.
3. The test is limited to the detection of AMH concentration in human serum or plasma. The use of other specimen types has not been validated.
4. A clinical diagnosis should not be based on the results of a single test and should only be made by the physician after all clinical and laboratory findings have been evaluated.
5. If the OD value of a specimen is greater than that of the highest calibrator, it is recommended to dilute the specimen with AMH Calibrator C1 and test again.
6. Any interpretation or use of this test result must also integrate other clinical findings as well as the professional judgment of health care providers.

REFERENCES

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Index of Symbols

Consult instructions for use	REF Catalogue number	Use-by date
IVD For *in vitro* diagnostic use only	LOT Batch code	Tests per kit
2°C 8°C Temperature limit 2-8°C	Do not re-use	Keep dry
Manufacturer	Date of manufacture	If device is non-sterile
Do not use if package is damaged	Keep away from sunlight	Warnings / Precautions

Athenese-Dx Pvt. Ltd.
Module No. 407 & 408, 4th Floor,
TICEL Bio Park II, No. 5, CSIR Road.
Taramani, Chennai-600113, India
Tel: +91-44-22541131
E-mail: info@athenesedx.com
Website: www.athenesedx.com

PI-AE1240 Rev. C
Effective date: 06.01.2023
English version