Anti-Mullerian Hormone (AMH)

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for Quantitative Determination of Anti-Mullerian Hormone (AMH) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

AMH test is intended for the quantitative determination of Anti-Mullerian Hormone (AMH) levels in human serum. For In Vitro Diagnostic Use Only.

INTRODUCTION

Anti-Mullerian hormone is a glycoprotein hormone structurally related to inhibin and activin from the transforming growth factor beta superfamily, whose key roles are in growth differentiation and folliculogenesis. AMH expression is critical to sex differentiation at a specific time during fetal development and appears to be tightly regulated by nuclear receptor SF1, transcription GATA factors, sex-reversal gene DAX1, and follicle-stimulating hormone (FSH). AMH is activated by SOX9 in the Sertoli cells of the male fetus thereby arresting the development of fallopian tubes, uterus, and upper vagina. AMH is also a product of granulosa cells of the preantral and small antral follicles in women. As such, AMH is only present in the ovary until menopause. AMH level is also lower and even below the detection limit if women with premature ovarian failure of any cause, including after cancer chemotherapy, etc.

PRINCIPLE OF THE ASSAY

AMH Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. In this assay, standards and samples are directly incubated in micro titer wells which have been coated with anti-AMH antibody. After the first incubation and washing, another anti-AMH detection antibody labeled with horseradish peroxidase (HRP) is added to each well. After a second incubation and washing step, a "sandwich" of solid-phase antibody-human AMH-HRP-conjugated monoclonal antibody is formed. Following the substrate tetramethylbenzidine (TMB) is added to the wells. The reaction is stopped after specified time using stop solution and the absorbance is determined for each well using an ELISA reader. The enzymatic activity of the immunocomplex in each well is directly proportional to the amount of human AMH in the test samples. The AMH concentrations in the samples can then be calculated from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with monoclonal anti- AMH antibody
- AMH Sample Diluent. Ready to use.
- AMH Enzyme Conjugate (100X)
- AMH Conjugate Diluent
- TMB Substrate. Ready to use
- Stop Solution. Ready to use
- AMH Standard set of 6 standards labeled as A to F in Lyophilized form. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).
- Control Set in lyophilized form. For control range refer vial label.
- Pack Insert
- Plate Sealer
- Protocol Sheet
- Microwell Holder

Materials required but not provided:

- Precision pipettes: 10µl, 50-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **AMH** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to pink at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens, standards and controls should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

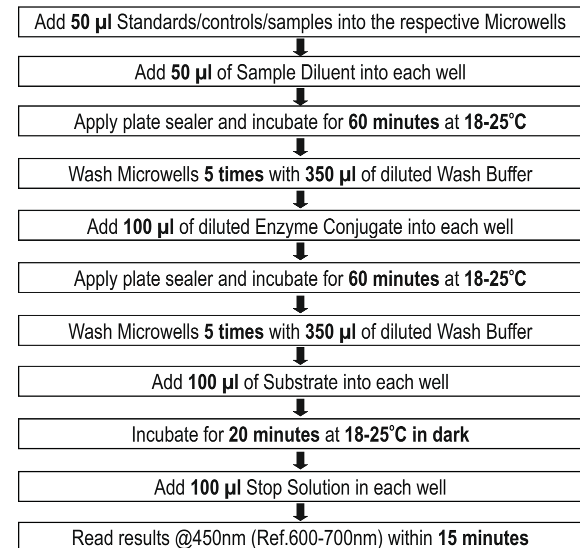
REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
3. Dilute enzyme conjugate with Conjugate diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.
4. Since the reference standards and controls are lyophilized, reconstitute each standard and control with 0.5ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards and controls should be sealed and stored at 2-8°C.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Enzyme Conjugate (µl)	10	20	30	35	45	50	60	70	75	85	90	100
Conjugate Diluent (µl)	1000	2000	3000	3500	4500	5000	6000	7000	7500	8500	9000	10000

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **50 µl** of standards, controls and sera into the appropriate wells.
2. Dispense **50 µl** of Sample Diluent into each well. Incubate at room temperature (18-25°C) for **60 minutes**.
3. After incubation, empty the microtitre wells and wash the plate **5 times** with **350 µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
4. Dispense **100 µl** of diluted Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for **60 minutes**.
5. After incubation, empty the microtitre wells and wash the plate **5 times** with **350 µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
6. Dispense **100 µl** of TMB Substrate into each well. Incubate at room temperature (18-25°C), in the dark, for **20 minutes**.
7. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
8. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.



CALCULATION OF RESULTS

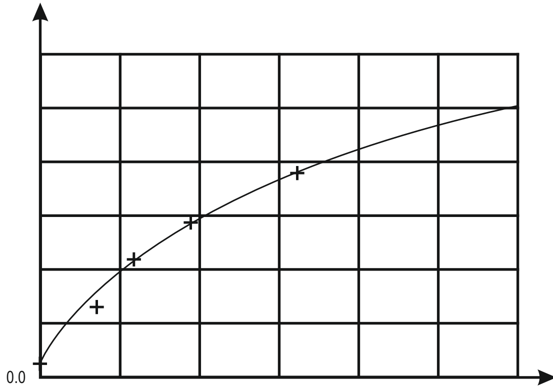
Construct a standard curve by plotting the absorbance obtained from each reference standard against its concentration in ng/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of AMH in ng/ml from the standard curve.

Example of Standard curve

Results of a typical standard run with optical density reading at 450nm (ref 600 – 700nm) shown in the Y axis against AMH concentrations shown in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

AMH (ng/ml)	Absorbance (450nm)
A	0.036
B	0.081
C	0.202
D	0.711
E	1.678
F	2.339



This Standard curve is for the purpose of illustration only, and should not be used to calculate samples. Each user should obtain his or her own Standard curve and data.

Expected Ranges of values

We tested 33 male samples, aged from 19 to 69 years old. All the values of these male samples were within the range of 0.7 to 19 ng/ml. Total of 34 female samples, aged from 13 to 61 years old, were also tested. The AMH value of the 29 female samples aged from 13-45 were within the range of 0.9-9.5 ng/ml; while the value of the other 5 female samples aged above 45 were <0.1 ng/ml. **Each laboratory should establish its own normal range based on gender and age.**

Gender	Age	Range (ng/ml)
Males	< 24 months	14~466
	24months~12 years	7.4~243
	> 12 years	0.7~19
Females	< 24 months	<4.7
	24months~12 years	<8.8
	13~45 years	0.9~9.5
	> 45 years	<0.1

The minimum detectable concentration of this assay is 0.073 ng/ml.

PERFORMANCE CHARACTERISTICS

A) Precision data

Two concentration levels of AMH samples were used to determine the inter precision of AMH ELISA assay kit.

Concentrations	No. of testing	Range	SD	CV
Level 1	10	0.1-06	0.153	4.7%
Level 2	10	5-15	0.862	5.9%

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B) Internal Evaluation:

In an internal Study **AMH** was evaluated against commercially available licensed kit with 90 random clinical samples and **AMH** has demonstrated 100% clinical correlation with the commercially available licensed kit.

C) External Evaluation:

AMH ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **AMH** ELISA has demonstrated 100% correlation with the reference method.

*Data file Orchid Biomedicals (P) Ltd.

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards, controls and samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

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7. Data on file: Orchid Biomedical Systems (P) Ltd.

SYMBOL KEYS

Symbol	Meaning	Symbol	Meaning
	Temperature Limitation		Consult Instructions for use
	Manufacturer	IVD	*In vitro* Diagnostic Medical Device
	Use by	REF	Catalogue Number
	Date of Manufacture	LOT	Batch Number / Lot Number
	This side up	Σ	Contains sufficient for <n> tests
	Do not reuse		

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