Version 25-04/24

User's Manual

AMH ELISA Kit

REF **DEIA-Z00113V2**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The AMH ELISA is an enzyme immunoassay for the quantitative measurement of Anti-Müllerian Hormone (AMH) in serum or plasma (EDTA or lithium heparin plasma).

General Description

Anti-Müllerian hormone also known as AMH is a protein that, in humans, is encoded by the AMH gene.It inhibits the development of the Müllerian ducts (paramesonephric ducts) in the male embryo. It has also been called Müllerian inhibiting factor (MIF), Müllerianinhibiting hormone (MIH), and Müllerian-inhibiting substance (MIS). AMH is a protein hormone structurally related to inhibin and activin, and a member of the transforming growth factor-β (TGF-β) family. It is a dimeric glycoprotein that has a molar mass of 140 kDa. AMH is secreted by Sertoli cells of the testes during embryogenesis of the fetal male. In females, it is secreted by the granulosa cells of ovarian follicles. In mammals, AMH prevents the development of the müllerian ducts into the uterus and other müllerian structures. The effect is ipsilateral, that is each testis suppresses Müllerian development only on its own side. In humans, this action takes place during the first 8 weeks of gestation. AMH is expressed by granulosa cells of the ovary during the reproductive years, and controls the formation of primary follicles by inhibiting excessive follicular recruitment by FSH.

Principles of Testing

The AMH ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal (mouse) antibody directed towards a unique antigenic site of the AMH molecule. During the first incubation, the AMH in the added sample bind to the immobilized antibody. The simultaneously added enzyme conjugate, which contains an AMH antibody conjugated to horseradish peroxidase, binds to the AMH forming a sandwich complex. After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of color is proportional to the concentration of the analyte in the sample. A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

Reagents And Materials Provided

1. Microtiterwells, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-AMH antibody (monoclonal).
2. Standard (Standard 0 - 5), 6 vials, 1 mL each, ready to use;

Concentrations: 0.0 – 0.4 – 1.0 – 4.0 – 10 – 20 ng/mL

Conversion: 1 ng/mL = 7.14 pmol/L

The standards are calibrated against the following reference material: 1 st WHO International Reference Reagent, Mullerian Inhibiting Substance/Anti-Mullerian Hormone, NIBSC code: 16/190 Contain non-mercury preservative.

3. Control Low & High, 2 vials, 1 mL each, ready to use; For control values and ranges please refer to vial label or Certificate of Analysis. Contain non-mercury preservative.
4. Enzyme Conjugate, 1 vial, 14 mL, ready to use; Anti-AMH antibody conjugated with horseradish peroxidase; Contains non-mercury preservative.
5. Substrate Solution, 1 vial, 14 mL, ready to use; Tetramethylbenzidine (TMB).
6. Stop Solution, 1 vial, 14 mL, ready to use; Contains 0.5 M H_2SO_4, Avoid contact with the stop solution. It may cause skin irritations and burns.
7. Wash Solution, 1 vial, 30 mL (40X concentrated)

Materials Required But Not Supplied

1. A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
2. Calibrated variable precision micropipettes
3. Absorbent paper
4. Distilled water
5. Timer
6. Graph paper or software for data reduction

Storage

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 8 weeks if stored as described above.

Specimen Collection And Preparation

Note: Serum or plasma (EDTA or lithium heparin) can be used in this assay. Samples containing sodium azide should not be used in the assay. In general, it should be avoided to use hemolytic, icteric, or lipemic specimens.

Specimen Collection

Serum: Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.

Plasma: Whole blood should be collected into centrifuge tubes containing anticoagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 7 days at 2 °C to 8 °C prior to assaying. Specimens stored for a longer time (up to 3 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

If in an initial assay, a specimen is found to contain more analyte than the highest standard, the specimen can be diluted with Standard 0 and re-assayed as described in "Assay Procedure".

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µL sample + 90 µL Standard 0 (mix thoroughly)

b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Standard 0 (mix thoroughly).

Reagent Preparation

Wash Solution

Add distilled water to the 40X concentrated Wash Solution. Dilute 30 mL of concentrated Wash Solution with 1170 mL distilled water to a final volume of 1200 mL. The diluted Wash Solution is stable for 2 weeks at room temperature.

Assay Procedure

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 25 µL of each Standard, Control and sample with new disposable tips into appropriate wells.
3. Dispense 100 µL Enzyme Conjugate into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for 60 minutes at room temperature.
5. Rinse the wells 3 times with 400 µL diluted Wash Solution per well, if a plate washer is used.

- OR -

Briskly shake out the contents of the wells.

Rinse the wells 3 times with 300 µL diluted Wash Solution per well for manual washing. Strike the wells sharply on absorbent paper to remove residual droplets.

Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

6. Add 100 µL of Substrate Solution to each well.
7. Incubate for 15 minutes at room temperature.
8. Stop the enzymatic reaction by adding 50 µL of Stop Solution to each well.
9. Determine the optical density of the solution in each well at 450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended) with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

Calculation

1. Calculate the average optical density (OD) values for each set of standards, controls and samples.
2. Using graph paper, construct a standard curve by plotting the mean OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean OD value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4-Parameter Rodbard or 4-Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as> 20 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Optical Density (450 nm)
Standard 0 (0.0 ng/mL)	0.03
Standard 1 (0.4 ng/mL)	0.08
Standard 2 (1.0 ng/mL)	0.16
Standard 3 (4.0 ng/mL)	0.55
Standard 4 (10 ng/mL)	1.27
Standard 5 (20 ng/mL)	2.28

Detection Range

The range of the assay is between 0.062 ng/mL – 20.0 ng/mL.

Detection Limit

The detection limit of the ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Standard 0 and was found to be 0.044 ng/mL.

The Limit of Blank (LoB) is 0.044 ng/mL.

The Limit of Detection (LoD) is 0.052 ng/mL.

The Limit of Quantification (LoQ) is 0.062 ng/mL.

Specificity

The following substances were tested for cross-reactivity of the assay:

Substance	Added conc. (ng/mL)	Mean cross-reactivity (%)
AMH	0.40 – 10	100
Inhibin A	2.0 – 2000	0.03
Actvin AB	2.0 – 2000	0.27
LH	2.0 – 2000	0.18
FSH	2.0 – 2000	0.26
HCG	2.0 – 2000	0.12
TSH	2.0 – 2000	0.39
TGF-β1	2.0 – 2000	0.18
TGF-β2	2.0 – 2000	0.18
Prolactin	2.0 – 2000	0.38

No substantial cross-reactivity of the assay to structurally related substances is detected.

Precautions

1. This kit is for research use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution coloured. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (20 °C to 26 °C) before starting the test. Temperature will affect the optical density readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of

different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.

17. Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

Limitations

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.