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User's Manual

Human anti-Mullerian hormone(AMH) ELISA Kit

REF **DEIA-Z00113**

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

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

An antibody specific for human AMH is coated onto the wells of the microtiter plate. Samples and standards of human AMH are pipetted into the wells for binding to the coated antibody. After washing procedure to remove unbound compounds, A detection Antibody specific for human AMH is added to the wells. Following a wash to remove any unbound detection antibody, a goat anti-rabbits POD labeled antibody is added to the wells. Following a wash to remove any unbound second antibody-enzyme reagent, a TMB one-Step Substrate Reagent is added to the wells and color develops in proportion to the amount of human AMH bound in the initial step. The color development is stopped and the intensity of the color is measured. The absorbance is proportional to the concentration of AMH. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

Reagents And Materials Provided

1. Antibody coated wells: 1 plates of 96 breakable wells (12 strips x 8 wells) coated with anti-MHA monoclonal antibody and blocked.
2. Standard: A serial diluted standard (ng/ml): 2400, 600, 150, 37.5, 9.375, 2.34, 0.586, 0(blank). Spin before use.
3. The storage standard is 400 ng/μL (un-diluent).
4. Detection Antibody (200x): 70μL of 200x concentrated detection antibody, diluted with Diluent 2, Spin before use.
5. Second antibody (3000x): Goat anti-rabbits POD labeled antibody, 1:3000 diluted with Diluent 2. Spin before use.
6. Diluent 1: 12ml, used to diluent the Standard and samples.
7. Diluent 2: 2 bottle (12ml/bottle), used to diluent the Detection antibody and second antibody.
8. Wash Concentrate (50x): 2 bottle, 15ml/bottle of 50x concentrated detergent solution.

9. TMB One-Step Substrate Reagent: 2 bottle, 15ml/bottle of TMB Substrate reagent.
10. Stop Solution: 2 bottle, 15ml/bottle of 0.18M acid buffer.
11. Plate Sealers: 3 sheets with adhesive backing.

Storage

For more detailed information, please download the following document on our website.

Specimen Collection And Preparation

Preparation Of Samples:

Serum:

Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulants may require increased clotting time.

Plasma:

Whole blood should be collected by venipuncture and centrifuged immediately after collection.

Specimen Storage

Samples may be stored at 4°C if assayed within 24 hours; otherwise samples must be storedat -20°C or -80°C to avoid loss of bioactivity and contamination. Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

Assay Procedure

1) Bring all reagents and samples to room temperature prior to use. It is recommended that all standards and samples be run in duplicate. A standard curve is required in each assay run.
2) Pipette 100µL of each standard and sample into appropriate wells. Cover wells with plate sealer and incubate for overnight (18-22 hours) at 4°C. We can also try to incubate for 2-3 hours at 37°C.
3) Wash the plate 5 times, by filling each well with Wash Buffer (at least 300µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
4) Add 100µL of prepared Detector antibody to each well. Cover wells with plate sealer and incubate for 2 hour at room temperature.

Within 15 minutes prior to use, add required dilution 2 into a clean tube, then pipette required volume of Detection Antibody, vortex well. For a full 96-well plate, Add 60µL Detection Antibody in 12 ml Reagent Diluent 2.

5) Wash wells as in step 3.
6) Add 100µL of prepared labeled second antibody to each well. Cover wells with plate sealer and incubate for

2 hour at room temperature. Within 15 minutes prior to use, add required dilution 2 into a clean tube, then pipette required volume of Second Antibody, vortex well. For a full 96-well plate, add 4µl second antibody in 12ml Reagent Diluent 2.

7) Wash wells as in step 3.
8) Add 100µL of TMB one-Step Substrate Reagent to each well. Incubate plate (without Plate Sealer) for 20 minutes at room temperature in the dark.
9) Add 100µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10) Read absorbance at 450 nm without 30 minutes of stopping reaction If wavelength correction is available, subtract the optical density readingsat570 nm from readings at 450 nm.

Recovery

95%