# Human AMH ELISA Kit

ARG82797 Human AMH ELISA Kit is an Enzyme Immunoassay kit for the quantification of AMH in Human serum and plasma (EDTA, lithium heparin).

Catalog number: ARG82797

Package: 96 wells

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## INTRODUCTION

Anti-Müllerian hormone (AMH), also known as Müllerian-inhibiting hormone (MIH), 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 can also serve as a molecular biomarker for relative size of the ovarian reserve. In humans, this is helpful because the number of cells in the follicular reserve can be used to predict timing of menopause. In bovine, AMH can be used for selection of females in multi-ovulatory embryo transfer programs by predicting the number of antral follicles developed to ovulation. AMH can also be used as a marker for ovarian dysfunction, such as in women with polycystic ovary syndrome (PCOS). [Provide by Wikipedia: Anti-Müllerian hormone]

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for AMH has been pre-coated onto a microtiter plate. Standards, Controls or samples are pipetted into the wells and any AMH present is bound by the immobilized antibody. The simultaneously added antibody-conjugate specific for AMH to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of AMH bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of AMH in the samples is then determined by comparing the O.D of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date. Opened kits retain activity for 8 weeks if stored as described above.

| Component | Quantity | Storage information |
|---|---|---|
| Antibody Coated microplate | 8 X 12 strips | 4°C |
| Standard 0-5 (0, 0.4, 1.0, 4.0, 10, 20 ng/mL ) | 6 vials, 1 mL each (ready to use) | 4°C |
| Control low (1.025 ng/ml; acc. range: 0.72 – 1.33 ng/ml) | I mL (ready to use) | 4°C |
| Control high (9 ng/ml; acc. range: 6.3 – 11.7 ng/ml) | I mL (ready to use) | 4°C |
| Antibody Conjugate | 14 mL (ready to use) | 4°C |
| 40X Wash Buffer | 30 mL | 4°C |
| TMB substrate | 14 mL (ready to use) | 4°C (protect from light) |
| STOP solution | 14 mL (ready to use) | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

## TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-26°C).
- Unused wells must be stored at 2-8 °C in the sealed foil pouch and used in the frame provided.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 40X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.

- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

## SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Samples containing sodium azide should not be used in the assay.
4. Specimens should be capped and may be stored for up to 7 days at 2-8°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.
5. 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.

## REAGENT PREPARATION

- 1X Wash Buffer: Dilute 40X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 15 mL of 40X Wash Buffer into 585 mL of distilled water to a final volume of 600 mL) The 1X Wash Buffer is stable for up to 2 weeks at room temperature.

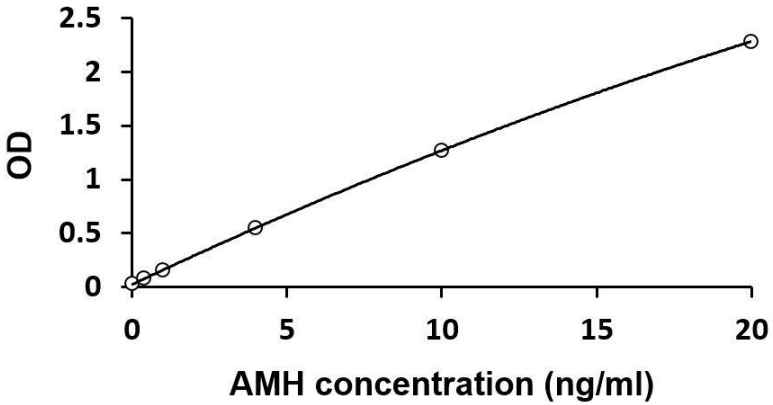
## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-26°C) before use. Standards and samples should be assayed in duplicates.

1. Add **25 µL** of **samples, Controls and Standards** to the **Antibody Coated microplate**.
2. Add **100 µL** of the **Antibody Conjugate** to each well. Thoroughly mix for **10 seconds**. **It is important to have a complete mixing in this step.**
3. Incubate at **RT** for **1 hour** on a microplate shaker.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with **1× Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Warm **TMB substrate** to **RT**. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate for **15 minutes** at room temperature in the dark.
6. Immediately Add **50 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 10 minutes** after adding the stop solution.

## EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the AMH ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
6. 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.
7. Conversion: AMH 1 ng/mL = 7.14 pmol/L

## QUALITY ASSURANCE

### Sensitivity

The limit of detection (LOD) of AMH is 0.052 ng/mL.

### Cross Reactivity

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

### Intra-assay and Inter-assay precision

The CV value of intra-assay was 2.4-7.3% and inter-assay precision was 3.1-6%

### Recovery

Samples have been spiked by adding AMH solutions with known concentrations. The recovery (%) was calculated by multiplying the ratio of measured and expected values with 100.

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Concentration (ng/mL) | 0.20 | 0.70 | 5.85 | 15.89 |
| Average Recovery (%) | 98.8 | 97.5 | 97.0 | 98.5 |
| Range of Recovery (%) From / To | 94.2 | 95.4 | 94.1 | 95.9 |
| | 101.8 | 100.3 | 101.1 | 100.8 |