For the quantitation of human AMH concentrations in cell culture supernatants, serum, and plasma.

## INTRODUCTION

Anti-Mullerian Hormone (AMH), also known as Mullerian Inhibitory Substance (MIS), is a dimeric glycoprotein protein that belongs to the Transforming Growth Factor-β (TGF-β) family. In females, AMH is produced by the granulosa cells of the antral and petrosal follicles in the ovary and plays a critical role in regulating folliculogenesis and ovarian function. AMH levels in women are used clinically to assess ovarian reserve, predict ovarian response to IVF stimulation, and diagnose conditions such as polycystic ovary syndrome.

The Fast Human AMH ELISA is a solid phase ELISA designed to measure human AMH levels in cell culture supernatants, serum, and plasma. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 1 hour, with no need for 5-6 hours (Fig. 1). The detection range is from 31 to 2000 pg/mL. The levels of human AMH samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human AMH protein.

## PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (see Fig. 1). A monoclonal antibody specific for human AMH is pre-coated onto a microplate. Standards or samples and a biotin conjugated detection antibody are pipetted into the wells and concurrently incubated to form a sandwich complex in one step. Simply aspirate each well without washing and directly add Streptavidin-HRP into the complex. Following a wash, an **ultra-sensitive TMB substrate solution** is added to the wells for color development. The color intensity is proportional to the amount of AMH bound in the initial step. The intensity of the color is measured by plate reading at 450 nm.

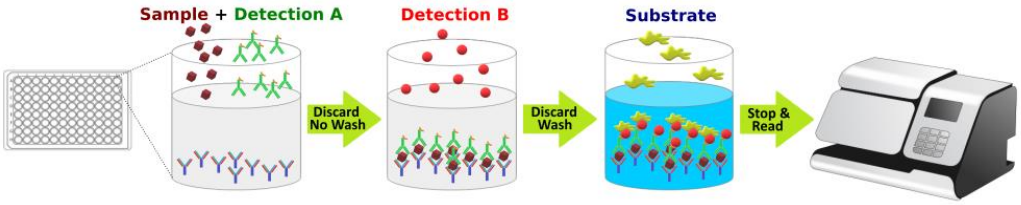



**Fig.1: Simple ELISA procedure**

## KIT CONTENT AND STORAGE CONDITIONS

| PART | PART# | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED |
|---|---|---|---|
| Human AMH Microplate | TBS3277A | 96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for human AMH. | Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C. |
| Human AMH Standard | TBS3277B | 30 µL of Recombinant human AMH protein (100ng/mL). | Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles. |
| Detection A | TBS3277C | 2.1 mL of human AMH antibody. | May be stored for up to 3 months at 2-8 °C. |
| Detection B | TBS3277D | 12 mL of Streptavidin-HRP | |
| Assay Diluent | TBS3277E | 12 mL of a buffered protein base with preservatives. | |
| 10x Wash Buffer | TBS3000W | 12 mL of concentrated solution (10x). | |
| TMB Substrate | TBS3000T | 12 mL of ultra-sensitive TMB substrate. | |
| Stop Solution | TBS3000S | 6 mL of 2 N sulfuric acid. | |

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

## PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

## REAGENT PREPARATION

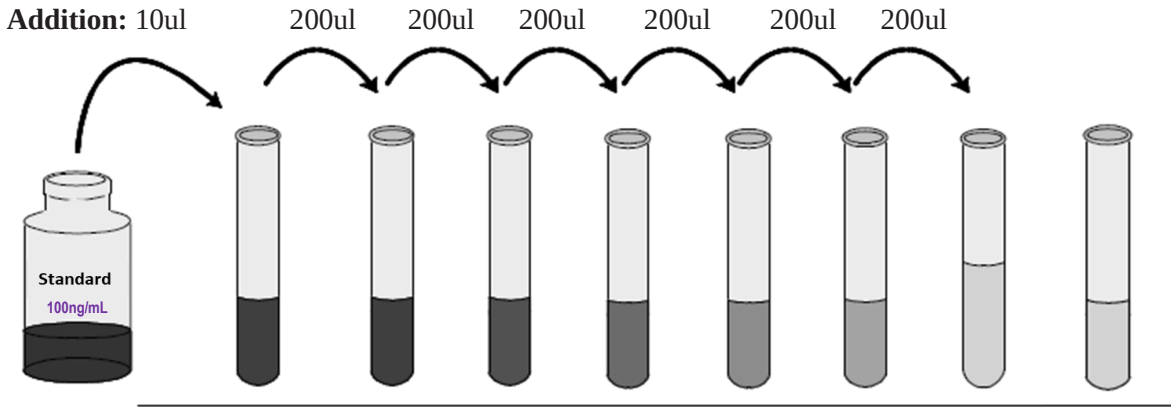
**Bring all reagents to room temperature before use.**

**Wash Buffer:** Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

**Human AMH Standard Preparation:**

1. Label test tubes as #1 through #8. Pipet 490 µL of 1x Assay Diluent into tube #1, and 300 µL into tubes #2 to #8 **as diagram below.**
2. Add 10 µL of the Human AMH Standard stock solution (100ng/mL) by dilution of 50X to tube #1 and mix.
3. Make 2x serial dilutions of the standard using the 2000pg/mL standard solution from tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 2000, 1000, 500, 250, 125, 62.5 and 31.3 pg/mL. Tube# 8 is Standard 0.

**Fig.2 Diagram for Human AMH standard preparation**




| Standard | Std1 | Std2 | Std3 | Std4 | Std5 | Std6 | Std7 | Std8 |
|---|---|---|---|---|---|---|---|---|
| Assay Buffer (µL) | 490 | 300 | 300 | 300 | 300 | 300 | 300 | 300 |
| Addition | Stock | Std1 | Std2 | Std3 | Std4 | Std5 | Std6 | |
| Addition Vol (µL) | 10 | 200 | 200 | 200 | 200 | 200 | 200 | 0 |
| Final Conc (pg/ml) | 2000 | 1000 | 500 | 250 | 125 | 62.5 | 31.3 | 0 |

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.**

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 60 min.**
3. Aspirate each well (***no wash***). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B** to each well. Incubate at **RT for 30 min.**
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue. If the color is light, the incubation time can be longer.

7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 5 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

## CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human AMH concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

## TYPICAL DATA

This standard curve ($R^2$=1.000) is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

## SENSITIVITY

The minimum detectable dose (MOD) of human AMH is typically 5 pg/ml.

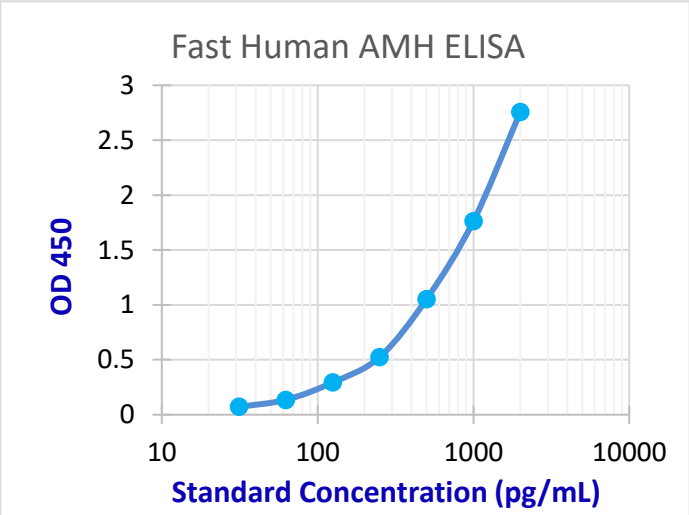
The Intra-CV is < 10%, the Inter-CV is < 12%.

## SPECIFICITY

This assay recognizes natural and recombinant human AMH.

## RELATIVE PRODUCTS

Human CEA ELISA (TBS3210)
Human AFP ELISA (TBS3212)
Human HE4 ELISA (TBS3213)
Human IL-1ß ELISA (TBS3219)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Human TNF-α ELISA (TBS3235)
Human IL-18 ELISA (TBS3239)

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