PromedeusLab

HUMAN ANTI-MÜLLERIAN HORMONE ELISA

Cat. No.: PL1002

Enzyme Immunoassay for the quantitative determination of Anti-Müllerian Hormone (AMH) in human serum and plasma.

Anti-Müllerian Hormone (AMH) is a glycoprotein belonging to the transforming growth factors (TGF-P). In females, AMH is secreted by granulosa cells of small follicles in the ovary.[1] Serum AMH levels strongly correlate with the number of growing follicles. Serum AMH levels are used in individualized follicle-stimulating hormone dosing protocols and may predict the risk of poor response or ovarian hyperstimulation syndrome.[3] Serum concentrations of AMH gradually decrease and fall below detectable levels in menopause. AMH is the best current available measure of ovarian reserve for different clinical conditions.[2]

Principle of AMH Elisa

The microtiter plate is coated with the antibody specifically binding the Anti-Müllerian Hormone. The human serum or plasma is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well. Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of AMH in the specimen. The concentration of AMH in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.

- **Add 100 µL of Standards, diluted QCs and Samples to the wells**

  Incubate for 1 hour at 25 °C, shaking at 300 rpm
- **3-times wash the wells (350 µL/well)**
- **Add 100 µL of Biotin-labelled Antibody to the wells**

  Incubate for 1 hour at 25 °C, shaking at 300 rpm
- **3-times wash the wells (350 µL/well)**
- **Add 100 µL of SAV-HRP to the wells**

  Incubate for 30 min at 25 °C, shaking at 300 rpm
- **3-times wash the wells (350 µL/well)**
- **Add 100 µL of Substrate Solution to the wells**

  Incubate for 10 min in the dark at 25 °C, NO shaking
- **Add 100 µL of Stop Solution to the wells**
- **Read the signal at 450 nm (450/630 nm) within 15 min**

Kit contents

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody	13 mL
Streptavidin-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	13 mL
Wash Buffer 15× conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

Material required but not supplied

1. Glassware and test tubes.
2. Microtiter plate washer.
3. Precision pipettes (various volumes) with tips.
4. Orbital shaker.
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation.

Warnings and precautions

1. For research use only.
2. For professional laboratory use.
3. The reagents with different lot numbers should not be mixed.
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25 °C ±2 °C).

Storage conditions

1. The kit must be stored at 2–8 °C.
2. The opened components can be stored for one week at 2–8 °C.

Preparation of reagents

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25 °C ±2 °C.

Preparation of Standards

Reconstitute lyophilized Human AMH Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min and dilute 1:4 prior to use. The concentration of human AMH in Master Standard is 3 ng/mL.

Prepare set of Standard solution as follows:

Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 3 ng/mL (lyophilized)	1000 µL	3 ng/mL
Std2	300 µL of Std1	300 µL	1.5 ng/mL
Std3	300 µL of Std2	300 µL	0.75 ng/mL
Std4	300 µL of Std3	300 µL	0.375 ng/mL
Std5	300 µL of Std4	300 µL	0.188 ng/mL
Std6	300 µL of Std5	300 µL	0.094 ng/mL
Blank		300 µL	0 ng/mL

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Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls with deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:4 in Dilution Buffer, prior to use, see Preparation of samples.

Preparation of Wash Buffer 1×

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15× conc. to 700 mL of deionized /distilled water (dH_2O). Mix well. Store at 4 °C for two weeks or at -20 °C for long term storage.

Preparation of samples

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70 °C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of samples is 1:4, i.e., for singlets 40 µL of sample + 120 µL of Dilution Buffer, for duplicates 80 µL of samples + 240 µL of Dilution Buffer, respectively.

Do not store the diluted samples.

Assay procedure

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for 1 hour at 25 °C ±2 °C, shaking at 300 rpm.
3. Wash the wells 3-times with 1× Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for 1 hour at 25 °C ±2 °C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for 30 min at 25 °C ±2 °C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for 10 min, at 25 °C ±2 °C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450 or 450/630 nm within 15 min.

Performance characteristics

Samples used in the tests were diluted 1:4 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human AMH giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.024 ng/mL of sample.

2. Precision

Intra-assay

Sample	Mean (ng/mL)	SD	CV (%)
1	1.9	0.1	7
2	3.4	0.2	5

Inter-assay (Run – to – run)

Sample	Mean (ng/mL)	SD	CV (%)
1	2.0	0.1	4
2	3.7	0.2	6

3. Accuracy

Dilution linearity

Sample	Dilution	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1		1.9	-	-
	2×	1.1	0.96	110
	4×	0.5	0.5	104
	8×	0.3	0.2	109
2		3.4	-	-
	2×	1.8	1.7	108
	4×	0.9	0.9	108
	8×	0.5	0.4	113

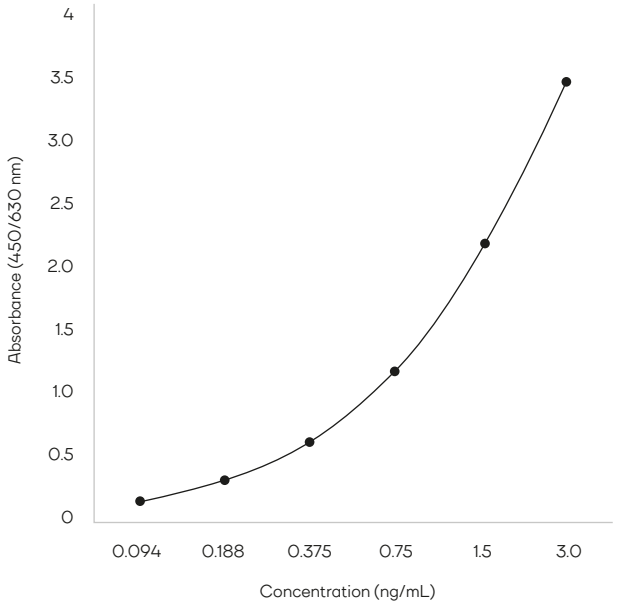
Spiking Recovery

Sample	Spike (ng/mL)	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1	-	1.2	-	-
	3.0	4.0	4.23	95
	1.5	2.7	2.75	96
	0.8	1.9	1.98	94

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.

Human AMH standard curve



Resources

1 Zec I, Tislaric-Medenjak D, Megla ZB, Kucak I. Anti-Müllerian hormone: a unique biochemical marker of gonadal development and fertility in humans. Biochem Med (Zagreb). 2011;21(3):219-30. doi: 10.11613/bm.2011.031. PMID: 22420235.

2 Broer SL, Broekmans FJ, Laven JS, Fauser BC. Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update. 2014 Sep-Oct;20(5):688-701. doi: 10.1093/humupd/dmu020. Epub 2014 May 12. PMID: 24821925.

3 Moolhuijsen LME, Visser JA. Anti-Müllerian Hormone and Ovarian Reserve: Update on Assessing Ovarian Function. J Clin Endocrinol Metab. 2020 Nov 1;105(11):3361–73. doi: 10.1210/clinem/dgaa513. PMID: 32770239; PMCID: PMC7486884.

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