AMH
ELISA TEST SYSTEM

INTENDED USE

The AMH ELISA Test System is intended for the quantitative measurement of AMH in human serum or plasma.

PRINCIPLE OF THE TEST

Monocent Inc. AMH ELISA is based on solid phase sandwich ELISA method. The samples and conjugate reagent (anti-AMH biotin & HRP) are added to the wells coated with Streptavidin. AMH in the patient's serum binds to the matched pair Abs, forming a sandwich complex and simultaneously the complex is being immobilized on the plate through streptavidin-biotin interactions. Unbound protein and HRP conjugate are washed off, through a washing step. Upon addition of the substrate, the intensity of color is proportional to the concentration of AMH in the samples. A standard curve is prepared by relating the color intensity to the concentration of AMH.

MATERIALS AND COMPONENTS PROVIDED

Microwells coated with Streptavidin	96 wells
AMH Standards: 6 vials (lyophilized)	1 ml each
AMH Controls: 2 levels (lyophilized)	1 ml each
AMH Conjugate Reagent: 1 bottle (ready to use)	12 ml
TMB Substrate: 1 bottle (ready to use)	12 ml
Stop Solution: 1 bottle (ready to use)	12 ml
20X Wash concentrate: 1 bottle	25 ml

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes
Disposable pipette tips
ELISA reader capable of reading absorbance at 450nm
Flat-head Vortex mixer
Plate shaker
Test tubes for sample preparation

STORAGE CONDITIONS

- Store the kit at 2 – 8 ˚C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- Do not expose reagent to heat, sun, or strong light.

PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate.
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8˚C) for 24 hours. If storage time exceeds 24 hours, store frozen at (-20˚C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENT PREPARATION

1. 20X Wash Buffer Concentrate: Prepare 1X wash buffer by adding the contents of the bottle to 475 ml of distilled water. Store 1X wash buffer at room temperature.
2. AMH Standards and Controls: Reconstitute the AMH standards and controls by adding 1ml of distilled water into each vial. Mix well until all the lyophilized content is reconstituted. The reconstituted standards and controls are stable at 4°C for a week; for longer storage, aliquot and freeze the standards and controls at -20°C.

TEST PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipette 50 µl of AMH standards, control, and patient's sera into appropriate wells.
3. Add 100µl of AMH Conjugate Reagent to all wells.
4. Cover the plate and incubate for 90 minutes at room temperature (20-25 ˚C) on a plate shaker (650rpm).
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature on a plate shaker (650rpm)
8. Add 50µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check AMH standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit.
2. To construct the standard curve, plot the absorbance for the AMH standards (vertical axis) versus the AMH standard concentrations in ng/ml (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
4. Value above the highest point of the standard should be retested after diluting with standard.

Example of Standard Data

	OD 450 nm	Conc. ng/mL
Std 1	0.012	0
Std 2	0.034	0.18
Std 3	0.099	0.7
Std 4	0.385	3
Std 5	1.406	11.5
Std 6	2.763	22

LIMITATIONS OF THE PROCEDURE

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following literature values for AMH may be used as initial guideline ranges only:

Female	
AGE	Expected Range, ng/mL
< 24 months	< 4.7
24 months - 12 years	< 8.8
13 - 45 years	0.9 - 9.5
> 45 years	<1 .0

PERFORMANCE CHARACTERISTICS

1. Precision

Intra-Assay

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	16	0.22	0.01	3.75
2	16	1.65	0.08	4.74
3	16	8.88	0.22	2.49

Inter-Assay

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	16	0.2	0.016	7.86
2	16	1.6	0.102	6.41
3	16	8.4	0.583	6.93

2. Sensitivity

Sensitivity of AMH SAV Microplate ELISA was assessed by running 24 replicates of "0"Standard in the assay and re-plotting the +2SD from the mean of off the dose response curve. The sensitivity of this ELISA was observed to be 0.018 ng/mL.

REFERENCES

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PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET076-2