Monobind Inc.
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AccuLite
CLIA Microwells

Anti-Müllerian Hormone (AMH) Test System
Product Code: 9775-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of AMH Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Anti-Müllerian Hormone (AMH) is a disulfide-linked homodimeric 140kDa glycoprotein from the trans-forming growth factor-β (TGF-β) superfamily of growth and differentiation factors. It is primarily produced by the gonads in both males and females.[1]

In fetal males, AMH is produced by the Sertoli cells and induces regression of the Müllerian duct and therefore promotes development of the male reproductive tract.[1] Infant males have very high levels of AMH (>30 ng/ml) that slowly decreases until post-pubescence where it remains at a low level (<10 ng/ml).[2-3]

In females, AMH begins to be produced near the time of birth with levels increasing until puberty. After puberty, blood AMH levels decrease until menopause where it becomes nearly undetectable (<0.1 ng/ml). AMH concentration in female blood has repeatedly been linked to ovarian reserve, thereby giving an indication to patients' remaining reproductive lifespans.[4] Additionally, high levels of AMH (>4.7 ng/ml, 80% CI) in females are an indication of polycystic ovarian syndrome (PCOS).[4]

When AMH levels drop below 1.0 ng/ml in females, they are considered to have low ovarian reserves. Patients in these ranges are advised to not delay family planning or to undergo infertility treatments such as in vitro fertilization.[4-5]

The AccuLite® AMH test kit is a highly sensitive assay that can be used to measure blood AMH levels in order to monitor progress of patients' infertility treatments and approximate the onset of menopause.

3.0 PRINCIPLE

Sandwich Equilibrium Method (TYPE 2):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of x-AMH antibody coated on the well.

Upon mixing the enzyme-labeled antibody (separate epitope) and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{AMH} + Ab_{(well)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{AMH} - Ab_{(well)}$$

$Ab_{(well)}$ = Antibody coated on well (Excess Quantity)
Ag_{AMH} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{AMH} - Ab_{(well)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

After sufficient time results, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. AMH Calibrators – 1.0 ml/vial (Lyophilized) Icons [A – F]
Six (6) vials of references for AMH at levels of 0(**A**), 0.2(**B**), 0.5(**C**), 1.0(**D**), 5.0(**E**) and 15.0(**F**) ng/ml. Store at 2-8 °C. ***Reconstitute each vial with 1.0ml of distilled or deionized water.*** The reconstituted calibrators are stable for 10 days at 2-8 °C. To store for a longer period, aliquot the reconstituted calibrators into cryo vials and store at -20 °C. ***DO NOT FREEZE/ THAW MORE THAN TWICE.*** A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation and are traceable against NIBSC code 16/190.

B. AMH Controls – 1.0 ml/vial (Lyophilized) Icons [M&N]
Two (2) vials of reference controls for AMH. Store at 2-8 °C. ***Reconstitute each vial with 1.0ml of distilled or deionized water.*** The reconstituted controls are stable for 10 days at 2-8 °C. To store for a longer period, aliquot the reconstituted calibrators into cryo vials and store at -20°C. ***DO NOT FREEZE/ THAW MORE THAN TWICE.*** A preservative has been added.

C. AMH Tracer Reagent – 6ml/vial – Icon
One (1) vial contains anti-AMH conjugate reagent. Store at 2-8 °C.

D. AMH Antibody Light Reaction Wells – 96 wells – Icon
One white 96-well microplate coated with x-AMH antibody. Store at 2-8 °C.

E. Wash Solution Concentrate – 20 ml/ vial – Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8 °C. *See Reagent Preparation section.*

F. Signal Reagent A – 7 ml/vial – Icon C[A]
One (1) vial containing luminol in buffer. Store at 2-8°C. *See Reagent Preparation section.*

G. Signal Reagent B– 7 ml/vial – Icon C[B]
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. *See Reagent Preparation section.*

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Do not expose reagents to heat, sun, or strong light.
Note 3: The above components are for one 96-well microplate

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate luminometer.
5. Test tubes for Signal Reagents A and B. (See Reagent Preparation)
6. Absorbent paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants for serum or EDTA/heparin containing tubes for plasma. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

If the specimen(s) cannot be assayed immediately after blood withdrawal, the sample(s) may be stored at temperatures of 2-8 °C for up to seven (7) days or -20 °C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing (a maximum of two freeze/thaw cycles prior to use). When assayed in duplicate, 0.100 ml (100 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days
2. **Working Signal Reagent Solution –** Store at 2 - 8°C.
   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacterial growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27 ℃).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8 ºC.**
2. Pipette 0.050 ml (50 µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of the AMH Tracer Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds, cover and incubate for 60 minutes at room temperature.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
7. Add 0.100 ml (100 µl) of working signal reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE PLATE AFTER SIGNAL ADDITION
8. Incubate at room temperature for five (5) minutes.
9. Read the relative light units (RLUs) in each well for 0.2 – 1.0 seconds. **The results should be read within thirty (30) minutes of adding the signal reagent solution.**

Note1: **Do not use the working signal reagent solution if older than 36 hours.**
Note 2: **Cycle (start and stop) mixing (4 cycles) for 5-8 seconds/cycle is more efficient than one continuous (20-30 seconds) cycle to achieve homogeneity. A plate mixer can be used to perform the mixing cycles.**
Note 3: **It is extremely important to accurately dispense the correct volume with a calibrated pipette and by adding near the bottom of the microwells at an angle while touching the side of the well**
Note 4**: For re-assaying specimens with concentrations greater than 15 ng/ml, the samples should be diluted and multiplied accordingly. Acceptable diluents are serum from postmenopausal females (<0.1 ng/ml AMH), the "0" calibrator, and other diluent solutions sold by Monobind.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of AMH in unknown specimens.
1. Plot the RLUs for each duplicate serum reference versus the corresponding AMH concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
2. Draw the best-fit curve through the plotted points.
3. To determine the concentration of AMH for an unknown, locate the average RLUs of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (37173) intersects the dose response curve at 5.54 ng/ml AMH concentration (See Figure 1).

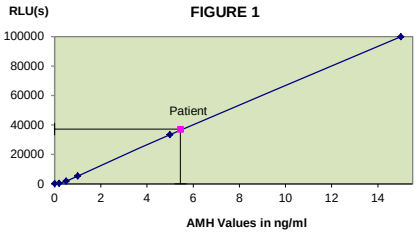
Note: Computer data reduction software designed for CLIA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	13	14	0
	B1	15		
Cal B	C1	423	417	0.20
	D1	411		
Cal C	E1	1896	1879	0.50
	F1	1863		
Cal D	G1	5319	5234	1.00
	H1	5150		
Cal E	A2	33416	33416	5.00
	B2	33953		
Cal F	C2	99798	100000	15.00
	D2	100202		
Patient	E2	38001	37173	**5.54**
	F2	36344		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

FIGURE 1



*If the RLUs readout is off-scale or higher than the average RLUs of the highest calibrator, sample should be repeated with dilution.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The dose response curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal solution initiates a kinetic reaction; therefore the reagents should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate luminometers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. pipettes, luminometers, washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. The reagents for AccuLite® CLIA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. *(Boscato LM Stuart MC.'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988:3427-33).* For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history, and, all other clinical findings.

13.0 EXPECTED RANGES OF VALUES

AMH levels were measured by the AMH AccuLite® Test System in apparently normal females of different age groups. The values obtained are given in Table 2.

Table 2
Female Reference Ranges for the AMH Test System

Age Group	N	Mean (ng/ml)	Median (ng/ml)	Minimum (ng/ml)	Maximum (ng/ml)
20-29	31	4.79	4.79	1.10	10.48
30-39	104	2.29	1.82	0.01	10.66
40-49	41	1.01	0.45	0.01	6.75

The expected values for males and females of untested age groups as determined by literature sources are collected in Table 3.[6]

Table 3
Additional Reference Ranges for the AMH Test System

Males	Range (ng/ml)
<24 months	14-466
2-12 years	7.4-243
>12 years	0.7-19
Females	
<24 months	<4.7
2-12 years	<8.8
13-19 years	0.9-9.5

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The precision of the AMH AccuLite® CLIA Test System was assessed on six levels of control pools. The mean, standard deviation, and CV% are given in the tables below.

Table 4
Precision data for the AMH Test System

Sample	Mean Value (pg/ml)	Within-Run Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%
Control 1	0.85	0.03	3.66	0.08	8.95
Control 2	4.99	0.14	2.85	0.34	6.72
Control 3	15.32	0.27	1.78	0.65	4.22
Patient 1	1.44	0.05	3.48	0.14	9.93
Patient 2	0.25	0.01	5.47	0.04	14.94
Patient 3	9.65	0.22	2.29	0.59	6.11

*As measured in forty experiments in duplicate over a 20 day period.

14.2 Sensitivity

The AMH AccuLite® CLIA Test System has a LoB = 0.0069 ng/ml, LoD = 0.0242 ng/ml, and LoQ = 0.109 ng/ml

14.3 Accuracy

14.3.1 Linearity

The linearity of the AMH AccuLite® CLIA Test System was tested by diluting human serum samples containing high levels of AMH (2-19 ng/ml) with human serum samples containing low levels of AMH (<0.2 ng/ml) or the "0" Calibrator. Observed values plotted against expected values produces excellent linearity throughout the range of the assay up to 19 ng/ml.

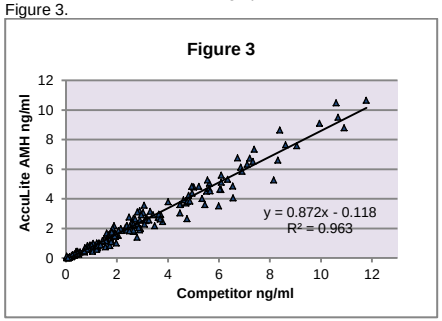
14.3.2 Recovery

The recovery of the AMH AccuLite® Microplate CIA Test System was calculated for five low AMH patient samples spiked with 0.5, 1.0, 2.0, 4.0, and 8.0 ng/ml AMH. Recoveries of all samples tested were calculated to be within 15% of the expected values.

14.3.3 Method Comparison

The AMH AccuLite® Microplate CLIA Test System was initially evaluated on 176 patients with known AMH concentrations calculated by a different AMH test system from a different manufacturer. Correlation between the two methods is excellent with a R^2 coefficient = 0.963. A graph of the data is shown in Figure 3.

Figure 3



14.4 Cross-Reactivity

The following analytes were tested and found to be non-reactive.

Analyte	Concentration	% Reactivity
Follicle Simulating Hormone	100 mIU/ml	<0.001
Human Chorionic Gonadotropin	1000 mIU/ml	<0.001
Leuteinizing Hormone	200 mIU/ml	<0.001
Prolactin	100 ng/ml	<0.001

15.0 REFERENCES

1. Wilson, J.D.; George, F.W.; Griffin, J.E. The hormonal control of sexual development. *Science* 1981, 211, *4488*, 1278-1284.
2. Jopling, H.; Yates, A.; Burgoyne, N.; Hayden, K.; Chaloner, C.; Tetlow, L. Paediatric Anti-Müllerian Hormone measurement: Male and Female reference intervals established… *Endocrinal Diab Metab.* 2018, 1, *4*, e00021.
3. Matuszczak, E.; Hermanowicz, A.; Komarowska, M.; Debek, W. Serum AMH in Physiology and Pathology of Male Gonads. *Int. J. of Endocrin.* 2013, 2013, Article ID 128907, 6 pgs.
4. Broer, S.L.; Broekmans, F.J.M.; Laven, J.S.E.; Fauser, B.C.J.M. Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. *Human Reproduction Update*, 2014, 20, *5*, 688-701.
5. Lee, J.Y.; Ahn, S.; Lee, J.R.; Jee, B.C.; Kim, C.H.; Seo, S.; Suh, C.S.; Kim, S.H. Reference Values for the Revised Anti-Müllerian Hormone Generation II Assay: Infertile Population-based Study. *J Korean Med Sci* 2017, 32, 825-829.
6. Test ID: AMH Antimullerian Hormone (AMH), Serum. https://www.mayocliniclabs.com/test-catalog/Clinical and Interpretive/89711 (accessed Aug 16, 2019).

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MP9775 **Product Code: 9775-*300***

Size		96(A)	192(B)
Reagent (fill)	A)	1.0ml (dry) set	1.0ml (dry) set
	B)	1.0ml (dry) set	1.0ml (dry) set
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (7ml)	2 (7ml)

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Glossary of Symbols
(EN 980/ISO 15223)