

Instructions for Use

Anti-Mullerian Hormone (CLIA)

[Product Name]

Anti-Mullerian Hormone (CLIA)

[Packing Size]

24×1 Tests/Pkg (Calibrators included)

60×1 Tests/Pkg (Calibrators included)

60×1 Tests/Pkg

[Intended Use]

AMH assay is a chemiluminescent immunoassay (CLIA) used to quantitatively measure anti-mullerian hormone (AMH) in human serum, plasma, and whole blood samples.

Anti Mullerian hormone (AMH) is a homodimeric glycoprotein with a molecular weight of 140KDa. It belongs to the transforming growth factor- β superfamily ^[1]. AMH is produced by testicular sertoli cells in males and follicular granulosa cells in females, and its main role is to inhibit the growth of Mullerian tubes ^[2]. In women, AMH can prevent premature ovarian follicles or oocyte excessive consumption ^[3], and AMH declines with age gradually, and its level is closely related to ovarian function ^[4]. Since the secretion of AMH is not regulated by hypothalamus and pituitary gland, the level fluctuates little in the whole menstrual cycle, which can predict the changes of ovarian function more early and more accurately ^[5]. AMH also has obvious advantages in monitoring ovarian reserve, diagnosing ovarian related diseases, evaluating the impact of cancer treatment on patients' ovarian reserve, and preventing complications of ovarian hyper-stimulation syndrome. It is a good marker for evaluating ovarian function and assisting in the diagnosis of ovarian related diseases ^[6-9].

[Principle of the Assay]

This assay takes the double-antibody sandwich format. The detection principle is described below:

- (1) Step1, Sample and anti-AMH antibody coated paramagnetic microparticle are combined and incubated, AMH in the sample will bind to anti-AMH antibody coated microparticle.
- (2) Step2, Acridinium-labeled anti-AMH antibody conjugate is added to the mixture and incubated.
- (3) After the second step, a magnet captures the microparticle, and then unbound substance is washed off. Add pre-trigger and trigger solution to the reaction mixture sequentially to initiate chemiluminescence reaction;
- (4) A photomultiplier tube is used to measure photons generated from the reaction. Signal is amplified exponentially. The count is proportional to AMH concentration in the sample. AMH concentration is determined by an internal calibration curve.

[Main Components]

Packing Size

| Packing Size | | Fill Volume | | |
|-----------------------|--------------------|---------------------------------------|---------------------------------------|----------------|
| | | 24×1 Tests/Pkg (Calibrators included) | 60×1 Tests/Pkg (Calibrators included) | 60×1 Tests/Pkg |
| AMH Reagent Cartridge | Microparticle (R1) | 24×50 μ L | 60×50 μ L | 60×50 μ L |
| | Conjugate (R2) | 24×100 μ L | 60×100 μ L | 60×100 μ L |
| AMH Calibrator | AMH Calibrator C1 | 1×1.0mL | 1×1.0mL | / |
| | AMH Calibrator C2 | 1×1.0mL | 1×1.0mL | / |

| | | | | |
|------------------|--|-------|-------|---|
| Calibration Card | Calibration curve and calibrator information | 1 pcs | 1 pcs | / |
|------------------|--|-------|-------|---|

Main Composition

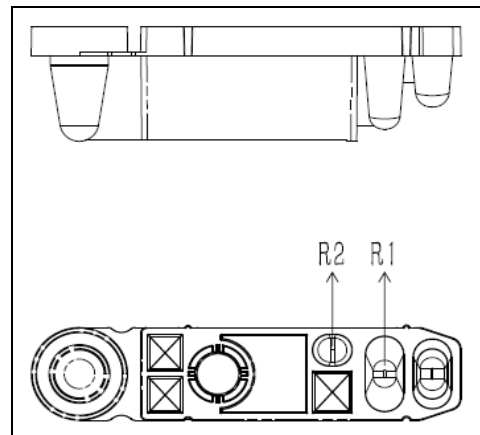
| Component | Main Composition | |
|-----------------------|---|---|
| AMH Reagent Cartridge | Microparticle (R1) | Mouse anti- AMH monoclonal antibody coated microparticle, ~0.3g/L; Tris buffer, 50mmol/L; ProClin 300, 0.5g/L |
| | Conjugate (R2) | Acridinium labeled mouse anti-AMH monoclonal antibody, ~200 μ g/L; MES buffer, 50mmol/L; ProClin 300 0.5g/L |
| AMH Calibrator C1 | AMH (recombinant);Tris buffer, 25mmol/L; ProClin 300, 0.5g/L | |
| AMH Calibrator C2 | AMH (recombinant); Tris buffer, 25mmol/L; ProClin 300, 0.5g/L | |
| Calibration Card | Calibration curve and calibrator information | |

Note: (1) Components in different lots of reagent cannot be mixed or exchanged for use.

(2) Traceability: This quantification method can be traced back to Roche Elecsys AMH assay.

(3) Information about calibrators can be found in the instrument after scanning the Calibrator Card (such as lot number and concentration etc.)

The position of each component is shown in the front view (Upper) and vertical views (Down) of the reagent pack.



Instruments and accessories needed but not supplied (available from Medcaptain)

- (1) Medcaptain Immu F6/F6S automatic chemiluminescent immunoassay analyzers;
- (2) Pre-trigger solution;
- (3) Trigger solution;
- (4) Washing solution;
- (5) 500 μ L pipette tips;
- (6) Sexual hormone controls;
- (7) Sample diluent.

[Storage and Shelf-life]

Storage: Store sealed reagent cartridge and calibrators at 2~8°C in upright position, and avoid freezing.

Shelf Life: 14 months.

Stability of Calibrators: Sealed vial of calibrators can be kept at 2~8°C in dark for 14 months. After calibrator C1 and C2 is uncapped, it can be stored at 10~30°C for 5 days, and at 2~8°C for 60 days.

The dates of manufacturing and expiration can be found on the labels.

[Matched Instruments]

Medcaptain Immu F6/F6S automatic chemiluminescent immunoassay analyzers

[Specimen Types]

Serum, plasma, and whole blood (collected with EDTA-K₂, EDTA-K₃, lithium heparin, and sodium heparin as the anti-coagulants) can be used in the testing.

Sample volume for each test: 50µL.

The collected sample should be tested as soon as possible.

Whole blood can be kept at 10~30°C for 4 hours, and it is recommended to complete the sample testing within 4 hours after sample collection.

Serum and plasma can be kept at 10~30°C for 8 hours, at 2~8°C for 5 days, and at -20°C or below for 6 months. Frequent freeze-thaw cycle should be avoided, and only one freeze-thaw cycle is allowed. If the sample contains precipitate or frozen floccule, centrifugation is needed to clear the sample before testing.

Sample collection tubes from different manufacturers may lead to variation in testing results, due to the difference in tube material and additives in the tubes. Medcaptain has not evaluated all types of sample collection tubes from different manufacturers. Each laboratory should make its own judgment about the usability of sample collection tubes.

[Test Procedure]**Reagent Preparation**

Reagent: AMH reagent cartridge (containing antibody coated magnetic microparticle R1, acridinium labeled antibody conjugate R2) is ready to use. It can be loaded directly into instrument after opening the package.

Calibrators: Calibrator C1 and C2 are ready for use. Each calibrator can be added to a sample cup, the cups can be loaded to a sample rack, and the rack can be pushed directly into instrument for calibrator testing.

Calibration

Refer to the operation manual of each chemiluminescent immunoassay analyzer for system calibration.

Calibration tests should be ordered before the first time use of AMH assay. Medcaptain provides AMH reagent pack and matched calibrators to calibrate the instrument.

Before calibration, scan the calibration card provided in the kit, and the calibration curve and calibrator information will be scanned into the system.

Before calibrator testing, take out reagent cartridges from the package, and load all of them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.);

Put calibrators on a sample rack, load the sample rack into the instrument.

On the screen interface of "Reagent > Request Calibration", select test name and lot number to request a calibration.

Select the position of each calibrator on the sample rack, set the number of repeat tests, start calibration.

Automatic immunoassay analyzer makes use of calibration test data to validate the calibration curve, and adjust the calibration curve automatically.

Instrument calibration is effective for 60 days.

A new calibration is needed in the following situation:

- (1) Change into a new lot of reagent;
- (2) Control test results are out of the target range;
- (3) The lot of reagent has been used on the same instrument for more than 60 days.

Refer to the Chapter of "Calibration" in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about calibration.

Control Testing

Sexual hormone controls are matched with AMH reagent pack. There are two levels of controls: Low Control (L) and High Control (H).

These two-level controls should be tested in accordance with any local applicable regulations. Control testing is highly recommended every time the lot of reagent has been changed, the instrument has been re-calibrated, or after trouble shooting/maintenance service.

Before control testing, take out reagent cartridges from the package, and load all of them into instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.);

Put controls on a sample rack, and load the sample rack into the instrument;

Select "Control" on the interface of test menu, select test name and control lot;

Click on Start, and begin the testing. Check the results after control test is finished.

Control testing results should fall into a specific range. If it is out of the target range, the user should check the system, such as expiration date of the controls, storage condition, instrument performance and status. After root cause analysis and correction, the user should test controls again. If the same problem exists, please contact customer service of Medcaptain.

Each laboratory should set up its own control range and frequency of control testing, based on its own practice.

Refer to the Chapter of "Control Testing" in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about control testing.

Sample Testing

Before sample testing, take out reagent cartridges from the package, and load all of them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.);

If a sample collection tube is directly loaded to the instrument for testing, the sample volume should be at least 1.0mL;

Uncap sample collection tubes, put samples on a sample rack, and push the sample rack into the instrument;

Select "Sample" on the interface of test menu, enter information of samples, select test name;

Click on "Start", and begin the testing. Check the results after sample test is finished.

The reagent usage for each test is: R1 50µL, R2 100 µL; the instrument aspirates and mixes each component in the reagent cartridge, and incubate at 37°C. Time duration from sampling to result is about 15 min.

Refer to the Chapter of "Sample Testing" in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about sample testing.

Result Calculation

Based on the built-in calibration curve, the instrument automatically calculate AMH concentration in each sample, in a unit of ng/mL

[Reference Intervals]

Samples for the study of reference intervals come from local area in Guangdong Province. A total of 948 healthy and normal people (Male: 148; Female: 800) and 125 PCOS women have been recruited, age distribution: 20~50. Serum tests give a reference interval of 2.5%~97.5% population as follows:

| Population | Age | Number of People | 2.5 th perc. (ng/mL) | Median (ng/mL) | 97.5 th perc. (ng/mL) |
|---------------|-------|------------------|---------------------------------|----------------|----------------------------------|
| Healthy women | 20-24 | 122 | 1.211 | 4.024 | 11.635 |
| | 25-29 | 135 | 0.815 | 3.311 | 9.878 |
| | 30-34 | 142 | 0.565 | 2.813 | 8.135 |
| | 35-39 | 135 | 0.135 | 2.003 | 7.456 |

| | | | | | |
|-------------|-------|-----|-------|-------|--------|
| | 40-44 | 145 | 0.023 | 0.882 | 5.435 |
| | 45-50 | 121 | 0.008 | 0.192 | 2.705 |
| PCOS women | 20-43 | 125 | 1.862 | 6.823 | 18.920 |
| Healthy men | 20-50 | 148 | 0.775 | 4.785 | 14.535 |

Due to the differences in geography, race, sex, and age of tested population, the reference interval may vary in different laboratories. It is highly recommended for each clinical lab to establish its own reference intervals.

[Interpretation of Test Results]

The test data is for clinical reference only. It cannot be used as the only confirmatory evidence nor to eliminate the possibility of diseases. Clinical diagnosis of patients should take clinical symptoms, body sign, disease history, other lab test results, and treatment response into comprehensive consideration.

The measurement range of this assay is: 0.01~23 ng/mL. If AMH concentration is lower than LoD, it will be reported as <0.01 ng/mL; If AMH concentration is over the upper limit, it will be reported as >23 ng/mL.

For sample with AMH concentration of >23 ng/mL, sample diluent can be used to dilute the sample manually if more accurate result is needed, (a dilution factor of 1:2 is recommended). Test the diluted sample in duplicate.

When the instrument shows a warning sign of "SMPL", it means insufficient sample volume. Make sure enough sample is added for repeating the test. When the instrument shows a warning sign of "SMPJ", it means the sample probe has been blocked. Clean sample clot in the probe before repeating the test.

Some results are tagged with signs. Refer to the Chapter of "Result Signs" in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about results tagged with signs.

[Limitation of the Test Method]

The test data is for clinical reference only. It cannot be used individually as the evidence to confirm or eliminate the possibility of diseases.

There is no Hook effect for AMH sample with a concentration of ≤1400 ng/mL.

For endogenous interference substances with concentration less than the value shown in the table below, measurement error caused by the interference is within ±10%.

| Endogenous Interference substance | Concentration of interference substance |
|-----------------------------------|---|
| Total Protein | ≤12 g/dL |
| Bilirubin | ≤25 mg/dL |
| Hemoglobin | ≤500 mg/dL |
| Triglyceride | ≤1000 mg/dL |

For drug interference with concentration less than the value shown in the table below, measurement error caused by the interference is within ±10%.

| Drug | Concentration | Drug | Concentration |
|---------------|---------------|---------------------------|---------------|
| Acetaminophen | 200 mg/L | Levodopa | 20 mg/L |
| Ibuprofen | 500 mg/L | Doxycycline hydrochloride | 50 mg/L |
| Ampicillin | 1000 mg/L | Acetylsalicylic acid | 1000 mg/L |
| Ascorbic acid | 300 mg/L | Theophylline | 100 mg/L |
| Heparin | 5000 U | Cefoxitin | 2500 mg/L |

For cross reactant interference with concentration less than the value shown in the table below, none of the cross-reactions were detected.

| Cross reactant | Concentration | Cross reactant | Concentration |
|----------------|---------------|----------------|---------------|
| Activin A | ≤ 100 ng/mL | Activin B | ≤ 50 ng/mL |
| LH | ≤ 500 mIU/mL | Activin AB | ≤ 50 ng/mL |

| | | | |
|-----------|--------------|-----------|--------------|
| FSH | ≤ 500 mIU/mL | HCG | ≤1000 mIU/mL |
| Inhibin A | ≤100 ng/mL | Inhibin B | ≤ 50 ng/mL |
| TGF-β | ≤ 420 ng/mL | / | / |

Heterophilic antibodies in human serum may react with immunoglobulin in the reagent or sample, and interfere with immunoassay in vitro. More clinical or diagnostic information is needed to confirm disease diagnosis of patients.

Some patients have frequent contact with animals, or have been treated or diagnosed with mouse monoclonal antibodies. They may have generated heterophilic antibodies. For example, some patients under monoclonal antibody treatment may have human anti-mouse antibodies (HAMA) in blood circulation, leading to false positive or false negative results. Anti-interference components are added to this reagent formulation to minimize the impact of HAMA and ANA, but the problem may not be totally eliminated, and some sample testing may still be impacted. More clinical and diagnostic information is needed to make a solid conclusion.

Samples with the titer of no less than 1:1000 by anti-nuclear IgG test kit (indirect immune-fluorescence method) were studied in interference tests. It has shown less than ±10% error in the test results.

For RF at a concentration of less than 1500 IU/mL, and HAMA at a concentration of less than 120ng/mL, measurement error of AMH is within ±10%.

[Property and Performance]

1 Limit of Blank

LoB ≤ 0.007 ng/mL.

2 Limit of Detection

LoD ≤ 0.01 ng/mL.

3 Accuracy

Accuracy should meet at least one of the following criteria:

- Test the accuracy reference samples at two concentration levels multiple times. The relative deviation between the measurement result and the target value must not exceed 10.0%.
- Spike AMH of a known concentration into real samples at different levels. Spiked recovery should be 100±15%.

4 Linearity

Test AMH samples with concentration in the range of 0.01~23 ng/mL, the linearity correlation coefficient $r \geq 0.9900$.

5 Repeatability

Coefficient of variation (CV) for the test results of low (1±0.2 ng/mL), middle (5±1 ng/mL) and high (10±2 ng/mL) corporate reference sample is less than 8.0 %.

6 Lot-to-lot Variation

Coefficient of variation (CV) for the test results of low (1±0.2ng/mL) , middle (5±1 ng/mL) and high (10±2ng/mL) corporate reference sample with three batches of reagent is less than 10.0 %.

7 Accuracy of Calibrator Value Assignment

Use primary calibrators with assigned values from higher level measurement procedure, calibrate the immunoassay analyzer, and use the same lot of reagent to measure the value of each product calibrator. The measured value of Calibrator C1 and C2 has a relative deviation within ±10.0 % from its assigned value.

8 Homogeneity of Calibrators

8.1 Within-vial Homogeneity

Within-vial homogeneity of Calibrator C1 or C2 is represented with coefficient of variation, and $CV \leq 8.0 \%$.

8.2 Between-vial Homogeneity














Between-vial homogeneity of calibrator C1 or C2 is represented with coefficient of variation, and $CV \leq 5.0 \%$.

[Attention Notes]

- It is for in-vitro diagnosis only.

- 2 It can only be used by professionals.
- 3 Never use expired reagent kit.
- 4 Never mix components from different kits, or from different reagent lots.
- 5 Do not put the reagent cartridges upside down.
- 6 Measurement of AMH in a sample using different detection systems may yield different results, due to the difference in test methods, assay specificity, and factors of interference. The measured values from different systems should not be directly compared to avoid inappropriate clinical interpretation.
- 7 Strictly follow the protocol in the package insert, and operate according to the lab guidelines.
- 8 The test results can only be used for clinical reference. Clinical diagnosis of patients should take symptoms, body sign, disease history, other laboratory test results, and response to treatment for comprehensive assessment.
- 9 User should wear gloves and lab coat. Rinse with water if the skin is in contact with the reagent. Flush eyes with copious of water if eyes are in touch with the reagent, and see a doctor immediately.
- 10 Take all samples and reaction waste as potential biohazards. All waste must be handled following the local government regulation.
- 11 This product is a single-use cartridge. Reagent cartridges should be put back into refrigerator and stored at 2~8°C if they are not opened within the same day.

[Interpretation of Signs]

| | | | |
|--|---|---|---|
|  | Temperature limit. |  | Date of manufacturing |
|  | <i>In vitro</i> diagnostic medical device |  | Catalogue number |
|  | Batch Code |  | Consult instruction for use |
|  | Use-by Date |  | Authorized representative in the European Community |
|  | This way up |  | CE marking of conformity |
|  | Manufacturer |  | Unique device identifier |
|  | Biological risks | | |

[References]

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[Basic Information]



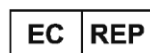
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