Instruction for Use

【Product Name】

Anti-Mullerian Hormone (eCLIA)

【Order Information】

REF NO.	Package Size
697043	50 T
697044	2×50 T
697045	100 T
697046	2×100 T

【Intended Use】

Immunoassay for in vitro quantitative determination of Anti-Mullerian Hormone (AMH) in human serum and plasma.

Anti - Mullerian Hormone (AMH), also known as Mullerian Inhibiting Substance (MIS). The molecular structure of AMH and inhibin, activin secreted by ovarian granular cells both belonging to one of members of the TGF -β (Transforming Growth Factor Beta) family, is responsible for regulating cell Growth and differentiation.AMH plays an important role in the development of fetal sex and is one of the important markers of gonadal function in both men and women.In males, AMH is mainly produced by testicular mesenchymal cells, starting from embryo formation and throughout life.During the development of the male fetus, AMH leads to degeneration of the Mullerian, forming a normally developing male genital duct. AMH serum concentrations are high before puberty[1]and then slowly decrease to postpuberty levels in females, AMH is mainly produced by ovarian granulosa cells.The concentration of AMH in serumgradually rises with age from infancy, reaching a peak at about 24.5 years[2] after puberty then gradually declines with age, and drops to an undetectable level after menopause[3].

Clinical serum AMH testing is mainly used to evaluate ovarian reserve function and predict the response to controll ovarian stimulation[4-5]. In adult women, serum AMH can be used to reflect the number of follicles (AFC), whose concentration is proportional to the number of AFC or ovarian reserve.With the increase of age, the continuous decrease of AMH concentration indicated that the number of follicles continued to decrease, indicating that the ovary continued to age.AMH concentration level is relatively stable during the menstrual period, which can be detected on any day of the menstrual period and is not affected by contraceptive drugs. It is clinically superior to FSH, LH and other direct indicators for evaluating ovarian reserve function[7].Studies have shown that AMH is a good indicator of reproductive aging and can be used to predict the age of menopause[6]. AMH has also been proposed as a new marker for the diagnosis of polycystic ovarian syndrome (PCOS), premature ovarian failure (POF) and other diseases[8].

【Principles of the testing method】

Sandwich principle. Total duration of assay: 18 minutes.

The Anti-Mullerian Hormone (AMH) Assay Kit adopts the double-site sandwich electrochemiluminescence method. Antigen in the sample, biotinylated AMH monoclonal antibody and the ruthenium (Ru) complex-labeled AMH monoclonal antibody form an antigen-antibody sandwich complex, which is bound to streptavidin coated magnetic particles, and then transferred to the measuring cell where the magnetic particles are captured onto the surface of the electrode, while the unbound substances are washed away. Chemiluminescence is generated after the electrode is electrified, and the generated optical signal is measured by a photomultiplier, processed by an instrument, and the concentration of AMH in the sample is calculated based on the calibration curve.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials (optional), and different components and reagents of lot numbers should not be used interchangeably.

Components	Ingredients	Volume (2×50 T)	Volume (50 T)	Volume (100 T)	Volume (2×100 T)
Magnetic Beads (MB)	Streptavidin-coated microparticles,0.75 mg/mL; 0.1MPBS; ProClin300	2×1.5 mL	1×1.5 mL	1×3.0 mL	2×3.0 mL
Reagent B (RB)	Anti-AMH-Ab~biotin, 1.5 μg/mL; 50 mM PBS; ProClin300	2×2.8 mL	1×2.8 mL	1×5.5 mL	2×5.5 mL
Reagent A (RA)	Anti-AMH-Ab~Ru(bpy)$_3^{2+}$, 0.5 μg/mL; 50 mM PBS; ProClin300	2×2.5 mL	1×2.5 mL	1×5.0 mL	2×5.0 mL
Calibrators	Horse serum, ProClin300; lyophilized	High: 1×2.0 mL Low: 1×2.0 mL			
Control Materials (Optional)	Horse serum, ProClin300; lyophilized	High: 1×2.0 mL Low: 1×2.0 mL			

The assignment process of supporting calibrators in this pack is traceable to Roche ELecsys AMH.

See the control material value sheet for the target values and ranges of control materials.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic).

Additional materials for Automated ECL Immunoassay Analyzer eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】

Store at 2° C-8℃. Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagents	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks
Stored on the analyzer	8 weeks

The calibrators and control materials are stable up to the stated expiration date.

Stability of the calibrators and control materials	
Lyophilized calibrators/control materials store at 2ºC~8ºC	18 months
Calibrators are stored at 2~8ºC after reconstitution	1 day
Control materials are stored at 2~8ºC after reconstitution	5 days
Reconstituted calibrators/control materials at -15ºC or lower than -15ºC	90 days (freeze-thawed only once)

The manufacturing date is labeled on the box, kit and bottles, and the expiration time are 18 months after production.

Damaged, expired or contaminated reagents should be discarded.

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, preparation and storage】

It is recommended to use human serum samples or plasma samples collected with EDTA-2K, EDTA-3K, heparin lithium, heparin sodium blood collection tubes.

Blood samples should be collected in accordance with the standard operation of venipuncture. After the sample is completely coagulated, centrifugation should be performed to remove residual cellular substances. The sample should be free of air bubbles during testing. Lipid layer covering the sample after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Samples can be stored for 24 hours at room temperature (18~28ºC), 5 days at 2~8ºC, and 6 months at -15ºC or below. Samples should avoid repeated freeze-thaw cycles. Do not use the samples after one freeze-thaw cycles.

【Testing Procedure】

Testing procedure and precautions

Before testing, the system operation manual of the measuring instrument should be carefully read, so as to obtain relevant information such as system operating procedures, sample management, safety precautions and maintenance, and materials required for testing should be prepared. AMH testing procedures should be called and set up in accordance with the system operating procedures.

Before using the reagent, put it into the analyzer 30 minutes in advance to automatically stir the magnetic bead particles and keep them in suspension.

Recommended environment temperature for testing: 10~30ºC; relative humidity: ≤ 80%.

The sample volume required for each AMH test is 50 μL.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) The reagents of different lot are used;

(2) The same lot of reagents were used on the analyzer beyond 4 weeks;

(3) Quality control findings outside the defined limits.

(4) The Buffer of different lots are used.

Pre-treatment of lyophilized calibrators:

Accurately add 2.0 mL of deionized water, and cover it at room temperature for 20 minutes to reconstitute the contents of the bottle. Mix well and avoid air bubbles. The calibrated product after mixing is marked and dispensed. If do not test immediately, quickly transfer to -25~-15ºC storage.

Quality Control procedure

For quality control, use AMH control materials.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality

control.
Please make sure that the correct values are used.
Pre-treatment of lyophilized control materials:
Accurately add 2.0 mL of deionized water, and cover it at room temperature for 20 minutes to reconstitute the contents of the bottle. Mix well and avoid air bubbles. The control product after mixing is marked and dispensed. If do not test immediately, quickly transfer to -25~-15ºC storage.

Calculation

The system software can automatically calculate the analyte concentration, with the result in pmol/L or ng/mL.
Conversion factors: pmol/L× 0.14 = ng/mL
ng/mL× 7.14 = pmol/L

Specimen dilution

AMH concentration in the sample higher than the upper limit of detection can be diluted with sample diluent.
The recommended dilution ratio is 1:9 (automatic dilution by instrument or manual dilution). If manual dilution, the result should be multiplied by the dilution ratio. If instrument automatic dilution, the instrument will automatically calculate the result.

【Biological Reference Interval】

By analyzing the serum samples of healthy people, it was calculated that the upper limit of the reference range of 2.5%~97.5% is as follows.

The crowd		Numbers	2.5th ~97.5th percentile(ng/mL)
Male		125	0.78-14.52
Female（years）	20-24	125	1.22-11.75
	25-29	123	0.892-9.85
	30-34	124	0.576-8.15
	35-39	126	0.147-7.50
	40-44	121	0.029-5.47
	45-50	120	0.01-2.71
PCOS Female		120	1.87-18.93

Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Interpretation of results】

When interpreting the test results, it is necessary to refer to the patient's overall clinical situation, including: symptoms, medical history as well as other corresponding data and information.

【Limitations】

The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.
The detection range of the kit is 0.01~25 ng/mL. If the AMH concentration in the sample is lower than the lower limit of detection, the result is reported as < 0.01 ng/mL; if the AMH concentration in the sample is higher than the upper limit of detection, then the sample should be diluted and re-tested. The results are reported after multiplying with the dilution factor.
When jaundice (bilirubin) is ≤ 50 mg/dL, hemolysis (hemoglobin) is ≤ 500 mg/dL, lipemia (triglyceride) is ≤ 1000 mg/dL, biotin is ≤ 30 ng/mL, total protein is ≤ 50 mg/mL, and HAMA is ≤ 30 ng/mL in the sample, the interference deviation of the measured results is within ±15.0%. When the concentration of rheumatoid factor in the sample is ≤ 1000 IU/mL, the relative recovery of the test results is between 85.0% and 115.0%.
Samples containing 100 ng/mL Inhibin A, 100 ng/mL Activin A, 500 mIU/mL Luteinizing Hormone (LH), 500 mIU/mL Follicle Stimulating Hormone (FSH) are tested, and the AMH measured results are not greater than 0.01 ng/mL.
When the AMH concentration reaches 500 ng/mL, there is no high-dose hook effect; when the AMH concentration reaches 1000 ng/mL, the test results are not be affected by the hook effect.

【Analytical Performance】

Lower limit of measurement

Limit of Detection (LoD) ≤ 0.01 ng/mL.

Accuracy

The recovery rate of measuring Accuracy control materials should fall between 85% and 115%.

Linearity

Within the range of 0.01 ng/mL~25 ng/mL, the correlation coefficient (r) of the kit should not be less than 0.9900.

Within-run imprecision (repeatability)

The coefficient of variation (CV) ≤ 8.0%.

Between-lot imprecision

The coefficient of variation (CV) ≤ 10.0%.

Homogeneity of calibrators and control materials

In-bottle homogeneity: coefficient of variation (CV) ≤ 10.0%.
Between-bottle homogeneity: coefficient of variation (CV) ≤ 10.0%.

Accuracy of calibrators

The supporting calibrators of the kit are tested, the relative deviation of their test results is within ±15.0%.

Measured values of quality control

The measured results of supporting assigned quality control of the kit should be within the quality control range.

Method comparison

A comparison of the Lifotronic AMH assay (y) with the Roche Elecsys AMH reagent kit (x) using clinical samples gave the following correlations:
Number of plasma samples measured: 335
Linear regression:
Y=0.9858X-0.0388
r = 0.9933

【Precaution and Warning】

This kit is only used for in vitro diagnosis;
When using this kit, the relevant operation precautions of the laboratory must be observed;
The test results of this kit can only be used as clinical reference, the patient's clinical evaluation should be based on the patient's clinical symptoms/signs, medical history, other laboratory test results and treatment response, etc.;
Due to methodological reasons or antibody specificity, the results of the same sample tested with reagents of different manufacturers may be different. Therefore, results obtained with different kits should not be directly compared, so as to prevent wrong medical interpretation; it is recommended that the Laboratory Department indicate the characteristics of the reagents in the test report issued to the clinician. If the reagent type is changed during series monitoring, continuous testing should be conducted, and the results should be compared with the original reagent results in parallel to re-determine the baseline value;
This product contains animal-derived substances, and may have potential biological risks. All samples and reaction wastes should be treated as the source of infection, and all wastes must be disposed of in accordance with local regulations.
Any serious incident that would be occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours /spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant.

【Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date	IVD	In vitro diagnostic medical device
LOT	Batch code	Σ	Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limit		This way up
EC REP	Authorized representative in the European Community	CE	Indicates this device is in compliance with Europe Directive.
REF	Catalogue number	P/N	Material code
	Warning		Date of manufacture
UDI	Unique device identifier		

【References】

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【Manufacturer】

Shenzhen Lifotronic Technology Co., Ltd.
Lifotronic Tower, No. 8, Qiuzhi East Road, Guancheng Community, Guanhu Street, Longhua, 518110 Shenzhen, PEOPLE'S REPUBLIC OF CHINA
E-mail: inter-service@lifotronic.com

【European Representative】

Shanghai International Holding Corp. GmbH (Europe)
Eiffestrasse 80, 20537 Hamburg, Germany
Tel. +49-40-2513175 Fax. +49-40-255726

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