EPITOPE DIAGNOSTICS, INC.

For Reference use only

EDI™ Human Anti-Müllerian Hormone CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the quantitative measurement of Human Anti-müllerian Hormone (AMH) Level in Serum or Plasma.

REF CL0812R RUO 100,150, 250 +2 +8 °C

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of human Anti-Müllerian Hormone (AMH) levels in serum or plasma using the ECL100 or ECL25 Immunoassay analyzer.

For Research Use Only

SUMMARY OF PHYSIOLOGY

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein hormone with a molar mass of 140 kDA[4]. AMH binds to its type 2 receptor AMHR2 which is part of the TGF beta signaling pathway[6]. The primary function of AMH is contribution to growth differentiation as it plays a key role in sex differentiation during fetal development[1]. Additionally, AMH is produced by granulosa cells and regulates folliculogenesis inhibiting excessive follicular recruitment by FSH[5]. Clinical applications may include aiding in the diagnosis of ovarian dysfunction[2,7]and ovarian reserve[3,8].

ASSAY PRINCIPLE

This CLIA is designed, developed, and produced for the quantitative measurement of human AMH level in serum samples. The assay utilizes a two-site “sandwich” technique with two antibodies that bind to different epitopes of AMH.

Assay calibrators, controls, or patient samples are added directly to a reaction vessel containing streptavidin coated magnetic particles. Simultaneously, anacridinium ester antibody and a biotin antibody are added. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of “magnetic particles– biotin AMH antibody –AMH–acridinium esterAMH antibody”.

The materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the trigger solution is added to the reaction vessel and light generated by the reaction is measured with the ECL100 or ECL25 analyzer. The relative light units (RLU) are proportional to the concentration of AMH in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in serum AMH concentration.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.Reagents from different kit lot numbers should not be combined or interchanged.
The following reagents are preloaded in the reagent cartridge:

1. AMH Magnetic Particle Solution (L0539)

Qty: 1 x 2.8 mL (100/kit), 2 x 2.5 mL (150/kit), 3 x 2.5mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use.

2. Biotin AMH Antibody (L0540)

Qty: 1 x 6 mL (100/kit), 1 x 9 mL (150/kit), 1 x 14mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use.

3. Acridinium Ester AMH Antibody (L0541)

Qty: 1 x 6mL (100/kit), 1 x 9 mL (150/kit), 1 x 14mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use.

4. EDI™ AMH Calibrators (L0544 – L0545)

Lyophilized Human Anti-Mullerian Hormone in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials
Storage: 2 – 8°C before reconstitution, <-20°C after reconstitution; Do not exceed 6 freeze-thaw cycles.
Preparation: Must be reconstituted with 0.5 mL of demineralized water and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely and there are no air bubbles prior to use.

5. AMH Controls (L0546 – L0547)

Lyophilized Anti-Mullerian Hormone in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials
Storage: 2 – 8°C before reconstitution, <-20°C after reconstitution; Do not exceed 6 freeze-thaw cycles.
Preparation: Must be reconstituted with 0.5 mL of demineralized water and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely and there are no air bubbles prior to use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for research use only. Source material which contains reagents of bovine serum albumin wasderived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ECL100 Immunoassay Analyzer or ECL25 Immunoassay Analyzer
2. CL011 Cuvettes (for ECL100) or CL010 Cuvettes (for ECL25)
3. Wash Reagent (P-594)
4. Trigger Solutions A and B (P-595)

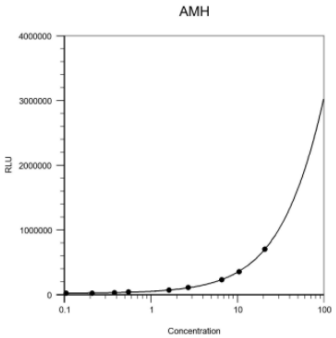
SPECIMEN COLLECTION AND PREPARATION

Serum or heparin plasma are acceptable samples. Collect whole venous blood into serum collection tubes (such as BD 366430) or tubes containing lithium heparin (such as BD 367880). Gently invert tube 3-4 times according to manufacturer's directions. Centrifuge tubes at 1500 RCF for 15 minutes. Carefully pipette off the serum or plasma and transfer to a clean test tube or vial.

1. Clinical samples should be tested within 2 hours after collection. If the measurement cannot be completed within 2 hours, please store under the following conditions:
   - storage at low temperature and away from light (2℃~8℃) for 2 days,
   - storage at -20℃ or below for 6 months
   - Freeze and thaw three times is acceptable.
2. Avoid heat-inactivated samples. Mixed, contaminated and hemolysis samples should be discarded.
3. Frozen samples should be restored to room temperature before testing. Due to possible volatilization, samples, calibrators and controls on the ECL platform should be tested within 2 hours.
4. Some substances in the samples will interfere with the test results. The common interfering substances and maximum allowable concentrations are as follows:
   - bilirubin 60 mg/dL
   - triglycerides 1500 mg/dL
   - hemoglobin 900 mg/dL
   - biotin 200 nmol/L
- For patients receiving high-dose biotin therapy (5 mg/ day), samples must be collected 8 hours after takingthe last dose of biotin
5. A single assay of this item requires 75 µL sample.This quantity does not include the amount of dead volume in the sample container, the capacity required for retesting, and other measurement items.For the definition of minimum required sample size, refer to the equipment manual.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration is required for the first time use of a reagent lot and every 14 days thereafter or when either kit control is out of range. Refer to appropriate system manuals for configuring calibrators.



QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random access format. Use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. We suggest performing a control test once every day. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator and control vials.
2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges.

2.2 For the ECL100, take out the Magnetic Particle bottle make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance. For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber. The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 OR ECL25 for instrument operation.

Components	Quality Control Hole (µL)	Sample Hole (µL)
AMH Controls (L0546, L0547)	75 µL	-
Patient Serum Samples	-	75 µL
Biotin AMH Antibody (L0540)	50 µL	50 µL
Acridinium Ester AMH Antibody (L0541)	50 µL	50 µL
AMH Magnetic Particle Solution (L0539)	25 µL	25 µL
IncubationPeriod 1		
Wash the reaction cup 3 times with the wash solution		
Trigger Solution A (P-595A)	200µL	200µL
Trigger Solution B (P-595B)	200µL	200µL

The assay total incubation time is less than 30 minutes.

INTERPRETATION OF RESULTS

The chemiluminescence analyzer calculates the concentration values of the sample and the control by a standard curve (fitting method: four parameters or point-to-point) and the measured RLU. Values are compared with the range of the marked value. If it exceeds the indicated quality control range, it indicates that the test is unqualified and needs to be re-tested.

Due to methodological differences or antibody specificity, there may be deviations between the test results of reagents from different manufacturers.Therefore, direct comparisons should not be made to avoid false interpretation.

EXPECTED VALUES

Mayo Clinic has suggested the following normal range for AMH and is summarized in the table below.

Reference Values	
Males	
< 24 months:	14.0 – 466 ng/mL
> 12 years	0.70 – 19.0 ng/mL
Females	
< 24 months	< 4.70 ng/mL
24 months – 12 years	< 8.80 ng/mL
13 – 45 years	0.90 – 9.50 ng/mL
> 45 years	< 1.00 ng/mL

AMH concentrations were established by measuring in one hundred sixty adult female (13-45) serum samples of normal healthy the USA population. The average AMH concentration range of this group was found to be **1 - 10 ng/mL**.It is recommended that all laboratories should establish their own normal range forAMH based on the local population.

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LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL25CLIA Immunoanalyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different lots cannot be mixed.
3. Test results from this product should not be the sole basis for clinical diagnosis.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measurement result is recalculated according to the dilution ratio to ensure the accuracy of the result.

PERFORMANCE CHARACTERISTICS

Hook Effect

The assay shows no hook effect up to 103 ng/mL.

LoB, LoD, LoQ

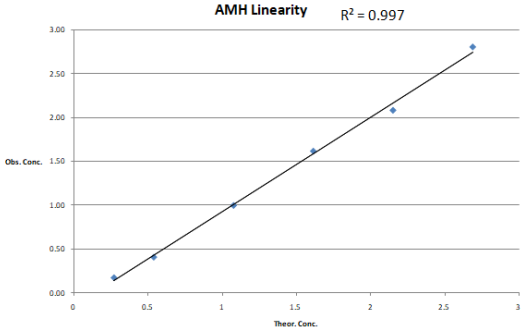
LoB, LoD, LoQ & Sensitivity were determined by running 5 blank samples as quadruplets followed by a titration of low level samples that approach the theoretical LoD.

- Limit of Blank (LoB) was determined by 20 replicates to be 0.071ng/mL.
- Limit of Detection (LoD) was determined to be 0.14ng/mL.
- Limit of Quantitation (LoQ) was determined to be 0.41ng/mL

Linearity

Linearity was determined by three replicates of each of the standards used to generate the multi-point calibration curve.

Standard	Average Concentration (ng/mL)	Theoretical Concentration (ng/mL)	CV (%)	R^2
1	0.21	0.269	8%	0.99
2	0.41	0.538	9%	
3	0.98	1.076	7%	
4	1.71	1.614	3%	
5	2.05	2.152	2%	
6	2.9	2.69	1%	



Repeatability

Reproducibility was determined by measuring ten replicates of controls.

Standard	Average Concentration (ng/mL)	CV (%)
P105 1:2	4.93	6%
P106 3:4	14.03	4%

Accuracy

Accuracy was determined by three replicates of two standards used to generate the multi-point calibration curve.

Standard	Average Concentration (ng/mL)	Target Value ± 15% (ng/mL)
P107	1.65	(1.46 – 1.97)
P108	6.38	(5.42 – 7.34)

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or to place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD In Vitro Diagnostic Device	RUO For Research Use Only	LOT Lot Number
REF Catalog Number	 Read instructions before use	 Number of Tests
 Store at	 Use by	 Keep away from heat and direct sun light
 Manufacturer	EC REP Authorized Representative in Europe	CE European Conformity