Hormone

ichroma™ AMH

INTENDED USE

ichroma™ AMH is a fluorescence immunoassay (FIA) for the quantitative determination of AMH (Anti-müllerian hormone) in human serum/plasma. It is useful as an aid in management and monitoring of premature ovarian insufficiency, menopause and ovarian reserve.

For *in vitro* diagnostic use only.

INTRODUCTION

AMH is a dimeric glycoprotein, also called müllerian inhibiting substance (MIS). AMH is a member of the transforming growth factor b (TGF-b) family of growth and differentiation factors.[1,2] In males, the major function of AMH is accountable for regression of the müllerian structures in utero. AMH is produced in the testicles until puberty and then slowly declines after puberty.[3] Release of AMH from the granulosa cells of antral follicles leads to measurable serum levels, and these concentrations have shown to be proportional to the number of developing follicles in the ovaries. Therefore, AMH was considered to be a marker for the process of ovarian ageing.[1]

AMH is an ideal marker for ovarian functional reserve because it is formed only by the primary follicles, which are potentially capable of maturation, and the secondary follicles. There is thus a very good correlation between the serum AMH level and the number of follicles potentially capable of maturation and thus also the ovarian functional reserve.[2] In women over 30 and particularly those over 35 years of age, AMH can be used as a screening test to assess fertility status.[3] As regards the rate of response to ovarian stimulation, AMH is of much greater value than inhibin B.[2] In addition, AMH is not subject to the same cycle-dependent fluctuations as inhibin B and FSH in the assessment of ovarian functional reserve. AMH can thus be used at any point during the menstrual cycle, whereas days 3-5 of the cycle should be selected when testing FSH and inhibin B.[4]

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized streptavidin on a test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for ichroma™ tests to show AMH concentration in the sample.

COMPONENTS

ichroma™ AMH consists of 'cartridges', 'detector tubes' and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has streptavidin at two test lines and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The detector has 2 granules containing anti AMH-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, anti-AMH-biotin conjugate, sodium azide as a preservative in Tris-HCl buffer. All detectors are packed in a box.
- The diluent contains tween 20 as a surfactant and sodium azide as a preservative in phosphate buffered saline (PBS), and it is pre-dispensed in a vial. The diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent, and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges or detector tubes. A cartridge should be used for testing one sample only. A detector should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge, detector tube, detector diluent, and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluent, and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector tube and the detector diluent contain sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure, low heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.

- No Biotin interference was observed in **ichroma™ AMH** when biotin concentration in the sample was below 2 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **ichroma™ AMH** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ AMH** should be used only in conjunction with instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Lithium heparin

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Disposable
Detector tube	2 – 30 °C	20 months	Disposable
Detector diluent	2 - 30 °C	20 months	Unopened
		3 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigens to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigens with time and/or temperature may also cause false negative result as it makes antigens unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-89

Components of **ichroma™ AMH**

- Cartridge box:
  - Cartridge 25
  - Detector tube 25
  - Detector diluent 1
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately with **ichroma™ AMH**.
Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M2** REF FPRR031
- **i-Chamber** REF FPRR009
- **Boditech AMH Control** REF CFPO-214

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ AMH** is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection when collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (serum, plasma) may be stored for up to a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, serum or plasma sample should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 2 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the contents of **ichroma™ AMH**: Sealed cartridges, detector tubes, a detector diluent, an ID chip, and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent as well as an ID chip.
- If the sealed cartridge, the detector tube, and the detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for ichroma™ tests.
- Turn on the i-Chamber and set temperature at 35 °C.
- Insert the ID Chip into 'ID Chip port'

※ **Please refer to the instrument for ichroma™ tests operation manual for complete information and operating instructions.**

CAUTION

- To minimize erroneous test results, we suggest that the ambient temperature of the cartridge should be 35°C during the reaction time after loading sample mixture to the cartridge.
- To maintain the ambient temperature to 35°C, you can use various devices such as an i-Chamber or an incubator and so on.

TEST PROCEDURE

▶ **ichroma™ II, ichroma™ M2**

1) Take 150 µL of detector diluent using a pipette and dispense it to the detector tube containing granules. When the granule forms are completely

dissolved in the tube, it becomes detection buffer. (The detection buffer must be used immediately. Do not exceed 30 seconds.)
2) Take 50 µL of sample (serum/plasma/control) using a pipette and dispense it to the detector tube.
3) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately. Do not exceed 30 seconds.)
4) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
5) Insert the sample-loaded cartridge into the slot of the i-Chamber or an incubator (35 °C).
6) Leave the sample-loaded cartridge in the i-Chamber or an incubator for 12 minutes.

⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*

7) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
8) Tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process. (ichroma™ M2 will start the test automatically after inserting.)
9) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
10) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ **ichroma™ III**

1) The test procedure is same with the 'ichroma™ II test procedure 1) ~ 4)'.
2) Insert the sample-loaded cartridge into the holder of ichroma™ III. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
3) Tap the 'Start' button on ichroma™ III to start the scanning process.
4) The cartridge goes inside and ichroma™ III will automatically start scanning the sample-loaded cartridge after 12 minutes.
5) Read the test result on the display screen of the ichroma™ III.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays AMH concentration of the test sample in terms of ng/mL.
- The working range: 0.02 – 15 ng/mL

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **ichroma™ AMH**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 0.014 ng/mL
  - Limit of Detection (LoD) 0.017 ng/mL
  - Limit of Quantitation (LoQ) 0.02 ng/mL
- **Analytical specificity**
  - **Cross-reactivity**
    Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ AMH** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Concentration
Activin A	100 ng/mL
Activin B	100 ng/mL
Inhibin A	50 ng/mL
Inhibin B	50 ng/mL
FSH	500 IU/L
LH	500 IU/L

  - **Interference**
    Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ AMH** test results did not show any significant interference with these materials.

Interferents	Concentration
Heparin	100 U/mL
Hemoglobin	5 g/L
Triglyceride	35 g/L
Bilirubin	300 mg/L
HAMA	2 µg/L
Albumin	65 g/L
Acetaminophen	1655 µmol/L
Ibuprofen	2425 µmol/L
Ampicillin	152 µmol/L
Acetylsalicylic acid	3.62 µmol/L
Ascorbic acid	528 µmol/L

- **Precision**
  - Single-site study
    Repeatability (within-run precision)
    within-laboratory precision (Total precision)
    Lot to lot precision
    3 Lots of **ichroma™ AMH** were tested for 21 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
AMH [ng/mL]	Repeatability		Within-laboratory precision		Lot to lot precision	
	AVG [ng/mL]	CV (%)	AVG [ng/mL]	CV (%)	AVG [ng/mL]	CV (%)
0.25	0.25	5.49	0.25	5.58	0.25	5.66
1.0	0.99	5.89	0.99	5.67	1.00	5.86
8.0	8.00	6.74	8.02	6.09	8.01	5.72

- Between person
  3 different persons tested 3 lots of **ichroma™ AMH**, 10 times at each concentration of the control standard.
- Between site
  1 person tested 1 lot of **ichroma™ AMH** at 3 different sites, 10 times at each concentration of the control standard.

AMH [ng/mL]	Between-person		Between-site	
	AVG	CV (%)	AVG	CV (%)
0.25	0.25	5.63	0.25	5.63
1.0	1.00	5.92	0.99	5.72
8.0	8.01	5.84	8.03	5.86

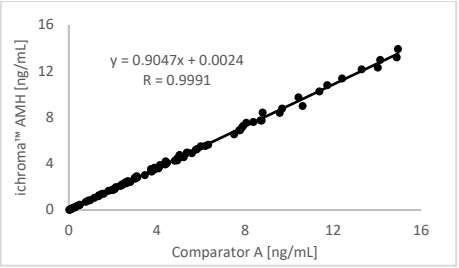
■ **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **ichroma™ AMH**. The tests were repeated 10 times at concentration of the control standard.

Expected value [ng/mL]	Lot 1	Lot 2	Lot 3	AVG	Recovery
0.05	0.05	0.05	0.05	0.05	101%
0.85	0.85	0.83	0.87	0.85	100%
1.65	1.62	1.60	1.67	1.63	99%
4.84	4.88	4.78	4.96	4.87	101%
6.43	6.52	6.38	6.60	6.50	101%
9.62	9.13	9.55	9.28	9.32	97%

■ **Comparability**

AMH concentration of 100 clinical samples were quantified independently with **ichroma™ AMH (ichroma™ II)** and **comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follow.



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Note: Please refer to the table below to identify various symbols

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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