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-antibody on test strip.

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

COMPONENTS

ichroma™ AMH consists of 'cartridges', 'detector tubes', 'detector diluent', 'ID chip' and an 'Instruction for use'.

- The cartridge contains the membrane called a test strip which has Streptavidin at the test line and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant in a box.
- The detector tube has 2 granules containing anti human AMH-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, anti-human AMH-biotin conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in as a preservative in phosphate buffered saline (PBS). All detector tubes are packed in a box.
- The detector diluent contains sodium azide as a preservative in phosphate buffered saline (PBS), and it is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instruction 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 tube 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.
- 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.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- 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 sample.

Recommended anticoagulant
Lithium heparin

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	4 - 30 °C	20 months	Disposable
Detector tube	2 - 8 °C	20 months	Disposable
Detector diluent	2 - 8 °C	20 months	Unopened
		12 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 antigen to the antibodies which is 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 antigen with time and/or temperature may also cause false negative result as it makes antigen 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 including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-89

Components of **ichroma™ AMH**

- Cartridge Box:
  - Cartridge 25
  - ID chip 1
  - Instruction for Use 1
- Buffer Box

✓ **For ichroma™ II**
- Detector tube (Capped with plastic lid) 25
- Detector diluent 1

✓ **For ichroma™-50**
- Detector tube (Sealed with aluminum foil) 25
- Detector diluent 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ AMH**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™-50** REF FPRR022
- **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.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- Samples 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.
- Serum or plasma sample stored frozen at -20 °C for 2 months showed no performance difference.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.
- Samples containing precipitates must be clarified by centrifugation.

TEST SETUP

- Check the contents of **ichroma™ AMH**: Sealed cartridges, detector tubes, detector diluent, ID chip, and Instruction 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 temperature for at least 30 minutes before testing.
- Turn on the i-Chamber and set temperature at 35 °C.
- It will take approximately 5-10 minutes to reach 35°C depending on environmental conditions.
- Turn on the instrument for ichroma™ tests.
  (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

- **ichroma™ II**

1) Transfer 150 µL of detector diluent using a pipette to detector tube containing a granule.
2) Transfer 50 µL (Human serum/plasma/ control) of sample using a pipette to the detector tube.
3) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 20 times. (The sample mixture must be used immediately.)
4) Pipette out 75 µL of a sample mixture and load it into the sample well on the cartridge.

5) Insert the sample-loaded cartridge into the slot of the i-Chamber (35°C) and leave the cartridge in i-Chamber for 12 minutes.
⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
6) 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.
7) Tap the 'START' button on the instrument for ichroma™ tests to start the scanning process.
8) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.
(Please refer to the ichroma™ II operation manual for complete information and operation instructions.)

■ **ichroma™-50**

1) Insert the tip array in the tip station.
2) Insert the detector array in the reagent station and cover the reagent station.
3) Open the diluent and insert the diluent in the diluent station.
4) Open the cover of the magazine station and pull and lift the cartridge magazine.
5) Insert the cartridges in the cartridge magazine one by one.
6) Insert the cartridge loaded cartridge magazine into the magazine station and close the cover of the magazine station.
7) Insert the sample tube into the blood collection tube rack and load the blood collection tube rack into the sampling station (loading part).
8) Tap the button which is provided in the upper side of the No. of test cartridge region to select ID chip what you want to use.
9) When the selected cartridge slot is activated, set the number of test cartridge by tapping.
10) Tap the button which provided in the upper side of the No. of reagent region to select ID chip what you want to use.
11) When the selected slot is activated, set the number of detector by tapping.
12) Set the number of pipette tips by tapping.
13) Tap the 'START' button on the left upper of the main screen to start test.
(Please refer to the ichroma™-50 operation manual for complete information and operation instructions.)

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 – 10 ng/mL
- Reference range
The following data is the AMH results measured by Elecsys AMH assay method. Serum samples were collected by female age group in a particular research.[8)]

No.	Age	N	No.	Age	N
1	20–24	150	5	40–44	142
2	25–29	150	6	45–50	169
3	30–34	138	7	PCOS women	149
4	35–39	138			

No	2.5th percentile (95% CI) ng/ml	5th percentile (95% CI) ng/ml
1	1.22 (0.478–1.67)	1.52 (0.758–1.810)
2	0.890 (0.493–1.21)	1.20 (0.797–1.750)
3	0.576 (0.256–0.958)	0.711 (0.256–1.120)
4	0.147 (0.053–0.474)	0.405 (0.053–0.496)
5	0.027 (0.010–0.063)	0.059 (0.017–0.119)
6	0.010 (0.010–0.010)	0.010 (0.010–0.010)
7	2.41 (1.67–3.01)	3.12 (2.29–3.77)

No	Median (95% CI) ng/ml	95th percentile (95% CI) ng/ml
1	4.00 (3.60–4.44)	9.95 (7.87–13.60)
2	3.31 (3.00–3.89)	9.05 (7.59–10.30)
3	2.81 (2.35–3.47)	7.59 (6.84–9.52)
4	2.00 (1.73–2.36)	6.96 (5.31–9.37)
5	0.882 (0.726–1.130)	4.44 (2.94–5.56)
6	0.194 (0.144–0.269)	1.79 (1.43–2.99)
7	6.81 (6.30–7.42)	12.6 (11.5–17.1)

No	97.5th percentile (95% CI) ng/ml
1	11.7 (9.11–15.70)
2	9.85 (8.91–11.30)
3	8.13 (7.27–9.72)
4	7.49 (6.49–10.90)
5	5.47 (3.92–6.76)
6	2.71 (1.79–4.16)
7	17.1 (13.3–20.3)

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.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with **ichroma™ AMH**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
(Please refer to the instruction 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 such as below the ones in the 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-reactivity material	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**

Interference materials such as below the ones in the table were added to the test sample(s) the same as the below concentrations. **ichroma™ AMH** test results did not show any significant interference with these materials.

Interference material	Concentration
Heparin	100 U/mL
Sodium Citrate	25.8 mg/mL
EDTA	2 mg/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**

- Repeatability (within-run precision)
  Repeatability of **ichroma™ AMH** was evaluated with results of 1 Lot.
- Total precision (within-laboratory)
  Total precision (within-run, between-run, between-day) of **ichroma™ AMH** was evaluated with results of 1 Lot.
- Lot to lot precision
  Lot to lot precision of **ichroma™ AMH** was evaluated with results of 3 Lots.
- Between person
  Three different persons tested one lot of **ichroma™ AMH**, ten times at each concentration of the control standard.
- Between site
  One person tested one lot of **ichroma™ AMH** at three different sites, ten times at each concentration of the control standard.

AMH	Between lot		Between person	
[ng/mL]	AVG	CV (%)	AVG	CV (%)
0.25	0.24	4.32	0.25	5.32
1	1.02	5.16	1.01	5.74
8	8.09	6.88	8.03	6.21
AMH	Between day		Between site	
[ng/mL]	AVG	CV (%)	AVG	CV (%)
0.25	0.25	5.31	0.25	4.38
1	1.02	4.96	1.03	6.05
8	8.13	6.11	8.08	7.03

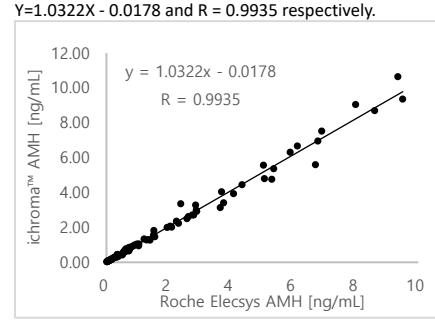
■ **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **ichroma™ AMH**. The tests are repeated 10 times in each different concentration.

Expected value [ng/mL]	Lot 1	Lot 2	Lot 3	AVG	Bias (%)
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 90 clinical samples were independently with **ichroma™ AMH** and Cobas e411 (Roche Diagnostics Inc. Switzerland) as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=1.0322X - 0.0178 and R = 0.9935 respectively.



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

	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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