

Hormone

ichroma™ Cortisol

INTENDED USE

ichroma™ Cortisol is a fluorescence immunoassay (FIA) for the quantitative determination of cortisol in human whole blood/serum/plasma. It is useful as an aid in management and monitoring of concentration of cortisol.

For in vitro diagnostic use only.

INTRODUCTION

Cortisol is a potent hormone known as a glucocorticoid that affects the metabolism of carbohydrates, proteins, and fats, but especially glucose. Cortisol test is performed on patients who may have malfunctioning adrenal glands. Cortisol level normally rises and falls during the day. It peaks its highest level between 6 and 8 AM and gradually falls, reaching its lowest point around midnight. When cortisol level is measured, blood specimen is usually collected at 8 AM and again at 4 PM. It should be noted that normal values may be adjusted in individuals who have worked during the night and slept during the day for long periods of time.

PRINCIPLE

The test uses a competitive immunodetection method.

Cortisol in the sample binds to the fluorescence-labeled detector antibodies in buffer, forming the complexes as a sample mixture. They will migrate onto nitrocellulose matrix, which will interfere with the binding of the free fluorescence-labeled detector antibodies to the immobilized-BSA-cortisol conjugate on the test strip.

More cortisol in the sample will result in less free detection antibodies to accumulate, which lead to less fluorescence signal by the free fluorescence-labeled detector antibodies. This signal is processed by the instrument for ichroma™ tests to show cortisol concentration in the sample.

COMPONENTS

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

- The cartridge contains the membrane called a test strip which has cortisol-BSA conjugate and anti-mouse IgG at two test lines, and streptavidin 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 tube has a granule containing anti cortisol-fluorescence conjugate, BSA-biotin-fluorescence conjugate, and sodium azide as a preservative in phosphate buffered saline (PBS). All detector tubes are packed in a pouch.
- The detector diluent contains tween 20 as detergent, and 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 the 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 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 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 (NaN₃), and it 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™ Cortisol** when biotin concentration in the sample was below 500 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™ Cortisol** will provide accurate and reliable results subject to the below conditions.
 - **ichroma™ Cortisol** should be used only in conjunction with the instrument for ichroma™ tests.
 - Have to use recommended anticoagulant.

Recommended anticoagulant

K₂ EDTA, K₃ EDTA, Lithium heparin

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.

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.

MATERIALS SUPPLIED

REF CFPC-24

Components of **ichroma™ Cortisol**

- 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™ Cortisol**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests

- **ichroma™ Reader** **REF** FR203
- **ichroma™ II** **REF** FPRR021
- **ichroma™ III** **REF** FPRR037
- **ichroma™ M3** **REF** FPRR035

- **Printer** **REF** FPRR007

- **i-Chamber** **REF** FPRR009

- **Boditech Hormone Control** **REF** CFPO-95

- **Boditech Cortisol Control** **REF** CFPO-236

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Cortisol** is human whole blood/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 (whole blood, serum, plasma) may be stored for a week at 2-8°C prior to being tested. If testing will be delayed more than a week, samples (serum, plasma) should be frozen at -20°C.
- The samples (serum, plasma) stored frozen at -20°C for 3 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™ Cortisol**: 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.
- Insert the ID chip into the '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 25°C during the reaction time after loading sample mixture to the cartridge.
- To maintain the ambient temperature to 25°C, you can use various devices such as an i-Chamber or an incubator and so on.

TEST PROCEDURE

► **ichroma™ Reader, ichroma™ II, ichroma™ M3**

- 1) Take 150 µL of detector diluent using a pipette and dispense it to the detector tube containing a granule. When the granule form is 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 (whole blood) or 30 µL of sample (serum/ plasma/control) using a pipette and dispense it to the 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 (25°C).
- 6) Leave the sample-loaded cartridge in the i-Chamber or an incubator for 10 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) Press the 'Select' or tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
(ichroma™ M3 will start 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 '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 10 minutes.
- 5) Read the test result on the display screen of the instrument for ichroma™ III.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays cortisol concentration of the test sample in terms of nmol/L.

■ Reference range

Time	Reference range [nmol/L]
Morning	151.58 – 793.38
Afternoon	67.9 – 473.22

- Working range: 50 - 800 nmol/L
- 1 ng/mL = 2.76 nmol/L (SI unit)

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™ Cortisol**. 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)	5.07 nmol/L
- Limit of Detection (LoD)	6.28 nmol/L
- Limit of Quantitation (LoQ)	50.0 nmol/L

■ 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™ Cortisol** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Concentration
Cortisone	1,000 nmol/L
Corticosterone	1,000 nmol/L
Progesterone	100 nmol/L
Prednisone	100 nmol/L
Testosterone	1,000 nmol/L
Prednisolone	100 nmol/L
Deoxycortisol	100 nmol/L
DHEA	1,000 nmol/L
Dexamethasone	2,000 nmol/L

- Interference

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

Interferents	Concentration
D-glucose	600mM
L-Ascorbic acid	2mM
Bilirubin [unconjugated]	4mM
Hemoglobin	200g/L
Cholesterol	130mM
triglyceride mixture	100mg/ml

■ Precision

- Single-site study

Repeatability (within-run precision)
within-laboratory precision (Total precision)
Lot to lot precision

3 Lots of **ichroma™ Cortisol** were tested for 21 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study				
Cortisol [nmol/L]	Repeatability		Total precision	
	AVG [nmol/L]	CV (%)	AVG [nmol/L]	CV (%)
70	70.18	6.1	70.10	6.0
270	265.66	5.9	268.41	5.7
560	648.22	5.4	641.68	5.7

Single-site study		
Cortisol [nmol/L]	Lot to lot precision	
	AVG [nmol/L]	CV (%)
70	69.98	5.8
270	269.67	5.9
560	649.34	5.7

- Between persons

3 Lots of **ichroma™ Cortisol** were tested 10 times at each concentration of the control standard.

- Between sites

1 Lot of **ichroma™ Cortisol** was tested 10 times in 3 different sites at each concentration of the control standard.

- Between readers

1 Lot of **ichroma™ Cortisol** was tested 5 times with 3 different readers at each concentration of the control standard.

Cortisol [nmol/L]	Between persons		Between sites	
	AVG [nmol/L]	CV (%)	AVG [nmol/L]	CV (%)
70	69.54	6.0	69.57	5.8
270	270.12	5.8	271.04	6.0
560	650.87	6.1	641.20	7.1

Cortisol [nmol/L]	Between readers	
	AVG [nmol/L]	CV (%)
70	69.86	6.1
270	272.39	4.6
560	645.22	6.0

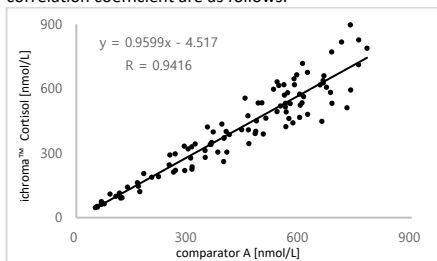
■ Accuracy

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

Cortisol [nmol/L]	Lot 1	Lot 2	Lot 3	AV	Recovery (%)
80	79.33	81.29	79.18	79.93	99.9%
220	220.14	223.66	217.96	220.59	100.3%
360	345.10	354.35	355.02	351.49	97.6%
500	503.43	488.16	496.42	496.00	99.2%
640	628.84	626.54	644.09	633.16	98.9%
800	815.38	816.27	763.15	798.27	99.8%

■ Comparability

Cortisol concentration of 100 clinical samples were quantified independently with **ichroma™ Cortisol (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 follows.



REFERENCES

1. Gustavo, E.T. Correlation between cortisol level and serotonin uptake in patients with chronic stress and depression. *Cognitive, Affective, & Behavioral Neuroscience* 2001, 1(4): 388-393.
2. Sonia, J.L., Mony, L., Susan, S., Antonio, A., Chaim, T., Mira, T., Bruce, S., M., Richard, L.H., and Michael, J.M. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nature* 1998, 1:69-73.
3. Bartels, M., Van den Berg, M., Sluyter, F., Boomsma, D.I., de Geus, E.J.C. Heritability of cortisol levels: review and simultaneous analysis of twin studies. *Psychoneuroendocrinology* 2003, 28:121-137.

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

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

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