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. **ichroma™ Cortisol** quantitatively measures the cortisol concentration of whole blood, serum and plasma.

## PRINCIPLE

The test uses a competitive immunodetection method.

The antigen 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 antigens 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 tube', '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-fluorescent conjugator,BSA-biotin-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline (PBS). All detector tubes are packed in a pouch.
- The detector diluent contains bovine serum albumin (BSA) as a stabilizer, tween 20 as detergent, 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 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 and 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 |
|---|
| $K_2$ EDTA, $K_3$ 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 antigen 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 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 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 | Unopened |
| Detector diluent | 2 - 30 °C. | 20 months | Unopened |
| | 2 - 30 °C. | 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 from **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.
- 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 sample (whole blood) or 30 µL (serum/plasma/control) using a pipette and dispense it to a tube containing the detection buffer. 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.)

3) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
4) Insert the sample-loaded cartridge into the slot of the i-Chamber or an incubator (25 °C).
5) 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.*

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) Press the 'Select' or tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
(ichroma™ M3 is tested automatically after inserting.)
8) The instrument for chrome™ tests will start scanning the sample-loaded cartridge immediately.
9) 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) ~ 3).
2) Insert the sample-loaded cartridge into the 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.
3) Tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
4) The cartridge goes inside the instrument for ichroma™ tests and 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™ tests.

## 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 | 151.6 - 793.3 |
| Afternoon | 67.9 - 473.1 |

- 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.

| Material | Conc. |
|---|---|
| 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.

| Material | Conc. |
|---|---|
| D-glucose | 600mM |
| Ascorbic acid | 2mM |
| Bilirubin [unconjugated] | 4mM |
| Hemoglobin (human) | 200g/L |
| Cholesterol | 130mM |
| triglyceride mixture | 100mg/ml |
| Biotin | 500 ng/mL |

- **Precision**
  - Repeatability (within-run precision)
  - Total precision (within-laboratory)
  - 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.

- Between persons
  Three different persons tested **ichroma™ Cortisol;** ten times at each concentration of the control standard.
- Between sites
  One person tested **ichroma™ Cortisol** at three different sites; ten times at each concentration of the control standard.
- Between readers
  Three different persons tested same lot of ichroma™ Cortisol with three different readers, five at each concentration of the control standard.

| conc. | Repeatability | | Total precision | |
|---|---|---|---|---|
| (nmol/L) | AVG | CV (%) | AVG | 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 |
| conc. | Lot to lot precision | | Between-person | |
| (nmol/L) | AVG | CV (%) | AVG | CV (%) |
| 70 | 69.98 | 5.8 | 69.54 | 6.0 |
| 270 | 269.67 | 5.9 | 270.12 | 5.8 |
| 560 | 649.34 | 5.7 | 650.87 | 6.1 |
| conc. | Between-site | | Between sites | |
| (nmol/L) | AVG | CV (%) | AVG | CV (%) |
| 70 | 69.57 | 5.8 | 69.86 | 6.1 |
| 270 | 271.04 | 6.0 | 272.39 | 4.6 |
| 560 | 641.20 | 7.1 | 645.22 | 6.0 |

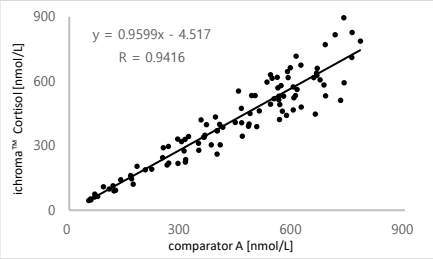
■ **Accuracy**

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.

| Expected value [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 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.

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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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