



# ichroma™ Calprotectin

## INTENDED USE

**ichroma™ Calprotectin** is a fluorescence Immunoassay (FIA) for quantitative determination of Calprotectin (MRP8/14; S100A8/S100A9) in **human feces**. It is useful as an aid in management and monitoring of the reflex gastrointestinal inflammation caused by several pathologies (inflammatory bowel disease, colorectal cancer and some enteropathies).

For *in vitro* diagnostic use only.

## INTRODUCTION

Calprotectin is a cytosolic protein present in neutrophils, whose concentration increases in the stool by Inflammatory Bowel Disease (IBD), specifically Crohn's disease and Ulcerative Colitis. The stability of Calprotectin to degradation keeps it stable in stools for up to seven days at room temperature and much longer periods at -20 °C. Calprotectin inhibits zinc-dependent enzyme systems, as a result, kills microbes and induces apoptosis in normal and cancer cells. In the presence of calcium, calprotectin is significantly resistant to proteolytic degradation and so is stable in stools keeps at room temperature for seven days. The fecal concentration of Calprotectin correlates with the histologic and endoscopic patterns of the intestinal inflammation in IBD patients.

## PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in the sample, forming antigen-antibody complexes, and migrate onto the nitrocellulose matrix to be captured by the other immobilized-antibody on the test strip.

The more antigen in sample, the more antigen-antibody complex are formed, which lead to a stronger intensity of the fluorescence signal from detector antibodies, which is processed by the instrument for ichroma™ tests to show the calprotectin concentration in the sample.

## COMPONENTS

**ichroma™ Calprotectin** consists of 'Cartridges', 'Extraction Buffer Tubes', 'ID chip' and 'Instruction For Use'.

- The cartridge contains a test strip, the membrane which has anti human calprotectin at the test line, with rabbit IgG at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The extraction buffer contains bovine serum albumin (BSA), detergent and sodium azide as a preservative in HEPES buffer.
- The extraction buffer is pre-dispensed in an extraction tube. 25 extraction buffer tubes are packaged in the cartridge box.

## WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- There should be no contamination with urine or water in samples.
- Samples should not be taken during menstruation, hemorrhoids or when using rectal medications.
- There should be no contamination with urine or water in samples.
- Lot numbers of all the test components (Cartridge, ID chip and extraction buffer) must match with each other.

- Do not interchange test components between different lots or use them after the expiration date, either of which might yield misleading test result(s).
- Do not reuse. An extraction buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge if found damaged or already opened.
- For shipping, samples must be packed in accordance with the regulations.
- Just before use, allow the cartridge, extraction buffer tube and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ Calprotectin** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used extraction buffer tubes, pipette tips and cartridges 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.
- **ichroma™ Calprotectin** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ Calprotectin** should be used only in conjunction with instrument for ichroma™ tests.

## STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The extraction buffer pre-dispensed in an extraction buffer tube is stable for 20 months if stored at 4-30 °C.
- 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. The non-responsiveness of antigens to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative 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-83

Components of **ichroma™ Calprotectin**

- Cartridge Box:
  - Cartridge 25
  - Extraction buffer 25
  - ID Chip 1
  - Instruction For Use 1

## MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

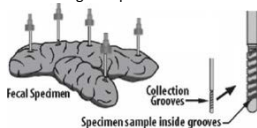
Following items can be purchased separately from **ichroma™ Calprotectin**. Please contact our sales division for more information.

- Instrument for **ichroma™** tests
  - **ichroma™ Reader** **REF** FR203
  - **ichroma™ II** **REF** FPRR021
  - **ichroma™-50** **REF** FPRR027
- **ichroma™ Printer** **REF** FPRR007

## SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Calprotectin** is **human feces**.

- Invert an extraction buffer tube and loosen the cap which is attached to a sampling stick (yellow in color).
- Introduce the sampling stick into the fecal sample six times at different sites. In order to get sampling even in the spiral groove of the stick and to ensure appropriate specimen to buffer ratio, try to avoid obtaining clumps of fecal matter.



- Return the stick to the extraction buffer tube. Tighten the cap thoroughly and shake the tube vigorously so as to disperse the specimen throughout the extraction buffer in the tube.
- If not to be used immediately after addition of the fecal sample, the extraction buffer tube should be refrigerated but must be analyzed using the test cartridge within 7 days.
- The collected specimen should be tested as soon as possible, but may be held up to 24 hours at 2-8 °C prior to testing. If testing will be delayed more than this time frame, samples should be frozen at -20 °C.
- Samples stored frozen at -20 °C for 6 months showed no performance difference.
- Repeated freezing and thawing can result in the change of test values.

## TEST SETUP

- Check the contents of **ichroma™ Calprotectin**: sealed Cartridge, Extraction Buffer Tubes and ID Chip.
- Ensure that the lot number of the cartridge matches with that of the ID chip as well as that on the extraction buffer tube.
- Keep the sealed cartridge (if stored in refrigerator) and the sample collection tube at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for **ichroma™** tests.
- Insert the ID Chip into the ID chip port of the instrument for **ichroma™** tests.
- Press the 'Select' button on the instrument for **ichroma™** tests. (Please refer to the 'Instrument for **ichroma™** tests Operation Manual' for complete information and operating instructions.)

## TEST PROCEDURE

### ► Instrument: **ichroma™-50**

- 1) Collect sample according to the sample collection method using a sampling stick in the 'sample collection and processing'. Then invert the extraction buffer tube again.
- 2) Insert pipette tips which are provided with **ichroma™-50** (or purchased on demand) into the tip station of **ichroma™-50**.
- 3) Insert test cartridges into the cartridge magazine and insert the cartridge.
- 4) Remove the cap of the extraction buffer tube and insert the extraction buffer tube into the sample rack provided with

**ichroma™-50**.

- 5) Insert test cartridges into the cartridge magazine and insert the cartridge-loaded magazine into the magazine station of **ichroma™-50**.
- 6) Input or set the number of tests that you want to perform and tap 'Start' button which is provided on the screen of **ichroma™-50**. (Please refer to **ichroma™-50** operation manual for complete information.)
- 7) **ichroma™-50** performs the tests automatically.
- 8) **ichroma™-50** will display the test results 10 minutes after loading samples.

### ► Instrument: **ichroma™ Reader/ichroma™ II**

- 1) Collect sample according to the sample collection method using a sampling stick in the 'sample collection and processing'.
- 2) Break off the black tip on the outside of the black cap.
- 3) Discard 3 drops of reagent onto the paper towel before applying the sample to the cartridge.
- 4) Hold the vial upside down and transfer 3 drops of the sample mixture and load it into the sample well on the cartridge.
- 5) Leave the sample-loaded cartridge at room temperature for 10 minutes.  
 ⚠ Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it may lead to a misleading result 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.
- 7) Press 'Select' button on the instrument for **ichroma™** tests to start the scanning process.
- 8) 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.

## INTERPRETATION OF TEST RESULT

- Instrument for **ichroma™** tests calculates the test result automatically and displays calprotectin concentration of the test sample in terms of mg/kg.
- **The cut-off: 50 mg/kg**
- **The Reference value:**

Value	Interpretation
<50 mg/kg	Negative
50 – 100 mg/kg	Borderline area, to be repeated (within 4-6 weeks)
>100 mg/kg	Positive

- In case of a positive result (above 50 mg/kg), consult a physician to discuss the test result. The physician may decide further course of action.
- Working range : 10-1,000 mg/kg

## QUALITY CONTROL

- Quality control test is 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 test 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™ Calprotectin**. 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) 2.91 mg/kg
  - Limit of Detection (LoD) 4.35 mg/kg
  - Limit of Quantification (LoQ) 10 mg/kg
- **Analytical specificity**
  - Cross reactivity

There was no significant cross-reactivity from materials listed below with the **ichroma™ Calprotectin** test measurements.

Cross-reactivity materials	Concentration
<i>Helicobacter pylori</i>	1.2 x 10 <sup>8</sup> CFU/mL
<i>Campylobacter jejuni</i>	1.2 x 10 <sup>8</sup> CFU/mL
<i>Candida albicans</i>	1.2 x 10 <sup>8</sup> CFU/mL
<i>Enterobacter cloacae</i>	1.2 x 10 <sup>8</sup> CFU/mL
<i>Escherichia coli</i>	1.2 x 10 <sup>8</sup> CFU/mL
<i>Pseudomonas aeruginosa</i>	1.2 x 10 <sup>8</sup> CFU/mL

- Interference

There was no significant interference from following materials with the **ichroma™ Calprotectin** test measurements.

Interference materials	Concentration
Human hemoglobin	2,000 µg/mL
Transferrin	4,000 mg/mL
Prednisolone	8.31 µmol/L
Ciprofloxacin	30.2 µmol/L
Stearic acid	0.4 mmol/L
Palmitic acid	6 mmol/L
Metronidazole	701 µmol/L
Vancomycin	69 µmol/L

- **Precision**
  - Between Lots

One person tested three different lots of **ichroma™ Calprotectin**, five times at each concentration of the control standard.

- Between persons

Three different persons tested one lot of **ichroma™ Calprotectin**, five times at each concentration of the control standard.

- Between days

One person tested **ichroma™ Calprotectin** during five days; five times at each concentration of the control standard.

- Between site

One person tested one lot of **ichroma™ Calprotectin** at three different sites, five times at each concentration of the control standard.

Calprotectin. (mg/kg)	Between-lot		Between-person		Between-day		Between-site	
	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)
10	9.70	10.04	9.86	10.90	9.78	5.57	9.95	9.02
50	48.50	5.69	50.16	6.56	49.52	1.87	50.22	6.27
500	495.63	3.66	493.26	3.37	498.33	3.44	501.59	3.10

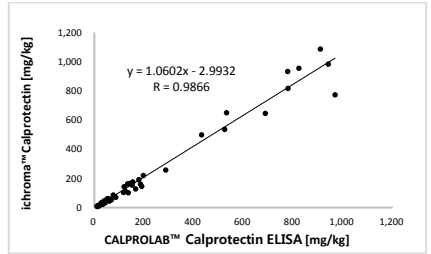
- **Accuracy**

The accuracy was confirmed by 3 different lots testing ten times each different concentration.

Calprotectin. (mg/kg)	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
10	10.47	9.46	9.70	9.88	99%
50	49.73	50.49	51.04	50.42	101%
500	493.96	500.90	506.58	500.48	100%

- **Comparability**

Calprotectin concentrations of 60 clinical samples were quantified independently with **ichroma™ Calprotectin** and CALPROLAB™ Calprotectin ELISA (Calpro AS, Norway) 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  $Y = 0.9973X + 0.5625$  and  $R = 0.9894$  respectively.

### REFERENCES

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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
	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

For technical assistance; please contact:  
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