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

More antigens in the sample will form more antigen-antibody complexes which lead to stronger intensity of the fluorescence signal by 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'.

- The cartridge contains the membrane called a test strip, which has anti human calprotectin at the test line, and rabbit IgG at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant in a box.
- The extraction buffer contains bovine serum albumin (BSA), calcium chloride, Triton X-100 as a detergent, and sodium azide as a preservative. All extraction buffers are pre-dispensed in each tube and packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- It is possible to use frozen samples. Please refer to "SAMPLE COLLECTION AND PROCESSING."
- 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, extraction buffer, and ID chip) 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 incorrect test result(s).
- Do not reuse cartridges or extraction buffer tubes. A cartridge should be used for testing one sample only. An extraction buffer 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 found damaged or has already been opened.
- For shipping, samples must be packed in accordance with local regulations.
- Allow cartridge, extraction buffer 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, extraction buffer tubes 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.
- **ichroma™ Calprotectin** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Calprotectin** should be used only in conjunction the instrument for ichroma™ tests.

STORAGE AND STABILITY

	Storage condition	
Component	Storage Temperature	Shelf life
Cartridge	4 - 30 °C	20 months
Extraction Buffer	4 - 30 °C	20 months

- 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 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 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-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

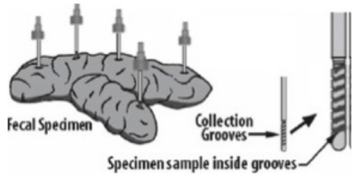
Following items can be purchased separately from **ichroma™ Calprotectin**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™-50** REF FPRR022
- **Printer** REF FPRR007
- **Boditech Calprotectin Control** REF CFPO-211

SAMPLE COLLECTION AND PROCESSING

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

- Collect feces into a clean container.
- 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.

[Sample storage]

- Store the samples in an extraction solution tube.
- The sample storage period in the extraction solution is below.
  - Samples stored at the room temperature for 7 days showed no performance difference.
  - Samples stored frozen at 2~8 °C for 10 days showed no performance difference.
  - The storage period may vary depending on the status and type of feces.
  - Recommended for use on the same day right after sampling.

TEST SETUP

- Check the contents of ichroma™ Calprotectin: sealed Cartridges, Extraction buffer tubes, ID Chip and Instruction for use.
- Ensure that the lot number of the cartridge matches with that of the extraction buffer tube as well as an ID chip.
- If the sealed cartridge and the extraction buffer 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.
  (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

▶ **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 magazine into the magazine station of ichroma™-50.
4) Remove the cap(black) of the extraction buffer tube and insert the extraction buffer tube into the sample rack which is provided with ichroma™-50.
5) Input or set the number of tests what you want to perform and tap 'Start' button which is provided in the screen of ichroma™-50. (Please refer to ichroma™-50 operation manual for complete information.)
6) ichroma™-50 performs the tests automatically.
7) ichroma™-50 will display the test results 10 minutes after loading samples.

▶ **ichroma™ Reader / ichroma™ II / ichroma™ III**

[Multi mode]

1) Collect sample according to the sample collection method using a sampling stick in the 'sample collection and processing'.
2) Assemble the sample collector and the extraction buffer into one and shake it about 10~15 times.
3) Break off the black tip on the outside of the black cap.
4) Discard 3 drops of reagent onto the paper towel before applying the sample to the cartridge.
5) Hold the vial upside down and transfer 3 drops of the sample mixture and load it into the sample well on the cartridge.
6) 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 inaccurate 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 'Select' or Tap 'START' button on the instrument for ichroma™ tests to start the scanning process.
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.

[Single mode]

1) Collect sample according to the sample collection method using a sampling stick in the 'sample collection and processing'.
2) Assemble the sample collector and the extraction buffer into one and shake it about 10~15 times.
3) Break off the black tip on the outside of the black cap.
4) Discard 3 drops of reagent onto the paper towel before applying the sample to the cartridge.
5) Hold the vial upside down and transfer 3 drops of the sample mixture and load it into the sample well on the cartridge.
6) Inserting the device 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.
7) Press 'Select' or Tap 'START' button on the instrument for ichroma™ tests.
8) Cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 10 minutes.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

※ For detailed test methods by the equipment, please refer to the analyzer instruction for use.

INTERPRETATION OF TEST RESULT

- The 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 provided on demand 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) 2.475 mg/kg
  - Limit of Detection (LoD) 4.76 mg/kg
  - Limit of Quantification (LoQ) 10 mg/kg

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

Cross reacting material.	Conc. (mg/kg)		
	10	50	500
	Interference (%)		
Helicobacter pylori	1.81	-0.83	-0.86
Campylobacter jejuni	-1.11	-0.05	1.23
Candida albicans	-1.84	0.38	-1.89
Enterobacter cloacae	-1.62	-1.67	-2.58
Escherichia coli	0.39	-0.42	3.31
Pseudomonas aeruginosa	2.09	-1.33	-3.65

  - 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™ Calprotetin** test results did not show any significant interference with these materials.

Interference materials	Conc. (mg/kg)		
	10	50	500
	Interference (%)		
Human hemoglobin	1.03	-0.74	0.72
Transferrin	1.7	3.19	0.86
Prednisolone	2.67	0.25	-2.35
Ciprofloxacin	1.46	1.99	1.94
Stearic acid	-0.35	0.53	1.58
Palmitic acid	-0.66	-0.46	-1.28
Metronidazole	-2.67	-3.77	1.07
Vancomycin	-2.73	2.16	1.14
DMF	0.77	-2.67	2.11
DMSO	-3.69	-2.72	-1.51

- **Precision**
  3 Lots of **ichroma™ Calprotectin** were tested for 30days (10days per 1 Lot at 1 site by one operator). Each standard material was tested 2 times per day. For each test, each material was duplicated.
  - Repeatability (within-run precision)
    Repeatability of **ichroma™ Calprotectin** was evaluated with results of 1 Lot.
  - Total precision (within-laboratory)
    Total precision of **ichroma™ Calprotectin** was evaluated with results of 1 Lot.
  - Lot to lot precision
    Lot to lot precision of **ichroma™ Calprotectin** was evaluated with results of 3 Lots.

- Between persons
  Three different persons tested one lot of **ichroma™ Calprotectin**, ten times at each concentration of the control standard.
- Between sites
  One person tested one lot of **ichroma™ Calprotectin** at three different sites, ten times at each concentration of the control standard.

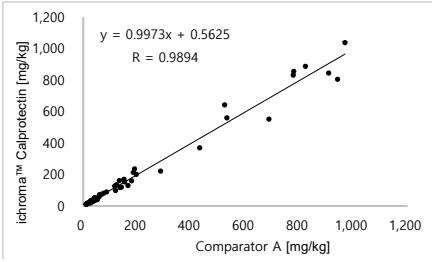
Conc.	Repeatability		Total precision	
[mg/kg]	AVG	CV(%)	AVG	CV(%)
25	23.77	4.4	23.70	4.2
50	49.25	4.6	48.94	5.0
250	245.83	3.8	246.33	3.5
Conc.	Lot to lot precision		Between-person	
[mg/kg]	AVG	CV(%)	AVG	CV(%)
25	24.90	5.6	25.19	6.9
50	50.46	6.1	50.66	5.2
250	248.93	4.5	248.27	5.2

Conc.	Between-site	
[mg/kg]	AVG	CV(%)
25	24.75	7.1
50	50.54	5.4
250	249.03	5.4

■ **Accuracy**
The accuracy was confirmed by testing 3 different lots, ten times at each concentration of the control standard.

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 (ichroma™-50)** and Comparator A 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 are as below.



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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

CE IVD