BIO TECHNOLOGY boditech

ichroma™

iFOB / Calp. Combo

INTENDED USE

ichroma™ iFOB / Calp. Combo is a fluorescence Immunoassay (FIA) for the quantitative determination of hemoglobin and calprotectin in human feces. It is useful as an aid in management and monitoring of colorectal cancer(iFOB) and inflammatory bowel disease (calprotectin).

ichroma™ iFOB / Calp. Combo is for *in vitro* diagnostic use only.

INTRODUCTION

Colorectal cancer is the third most common cancer in the world, with about 1 million new cases and more than 500,000 deaths per year. Screening methods for colorectal cancer include the immune-chromatography fecal occult blood (iFOB) test, barium enema, sigmoidoscopy. Large randomized controlled trials have shown that iFOB screening can result in decreased colorectal cancer mortality. The traditional FOB test used the chemical Guaiac, which is sensitive to Hb peroxidase activity. However, the Guaiac-FOB test has low sensitivity to clinically significant colorectal neoplasis and has low specificity due to its non-specificity for human Hemoglobin. To overcome these potential problems in immunochemical test, **ichroma™ iFOB / Calp. Combo** uses specific monoclonal antibodies against human hemoglobin.

Calprotectin is a cytosolic protein present in neutrophils, whose concentration in stool samples increases with 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 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 binds to antigen in the sample, forming antigen-antibody complex. These complexes then migrate onto the nitrocellulose matrix to be captured by other immobilized-antibodies on the test strip.

The more antigens in the sample, the more antigen-antibody complexes are formed, which leads to the stronger intensity of fluorescence signal from detector antibodies. This signal is processed by the instrument for ichroma™ tests to produce hemoglobin and calprotectin concentration in the sample.

COMPONENTS

ichroma™ iFOB / Calp. Combo consists of 'Cartridges', 'Extraction Buffer Tubes', 'ID chip' and 'Instruction For Use'.

- The cartridge contains a test strip, the membrane which has mouse monoclonal anti-hemoglobin and anti-calprotectin labeled with fluorescence and anti-rabbit IgG labeled fluorescence at the glaze line, mouse monoclonal anti-hemoglobin at the test 1 line, mouse monoclonal anti-calprotectin at the test 2 line, while 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 only and avoid the 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.
- Lot numbers of all the test components (Cartridge, ID chip and extraction buffer) 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 misleading of 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 is 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 at room temperature for over 30 minutes.
- **ichroma™ iFOB / Calp. Combo** 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™ iFOB / Calp. Combo** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ iFOB / Calp. Combo** 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 the antigen 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-84

Components of **ichroma™ iFOB / Calp. Combo**

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

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

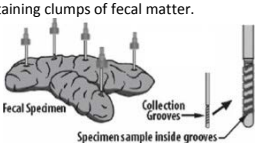
Following items can be purchased separately from **ichroma™ iFOB / Calp. Combo**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™-50** REF FPRR027

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ iFOB / Calp. Combo** is human feces.

- Invert an extraction buffer tube and loosen the cap attached to a sampling stick (yellow color).
- Introduce the sampling stick into the fecal sample five times at different sites. In order to get sampling evenly in grooves 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 extraction buffer in the tube.
- If not to be used immediately after addition of fecal sample, extraction buffer tube should be refrigerated but must be analyzed using the test cartridge within 7 days. If testing is expected be delayed for more than this, samples should be frozen at -20 °C.
- It is recommended that, once the specimen is dispersed in the extract buffer tube, the test be analyzed by the day's end.
- Repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the contents of **ichroma™ iFOB / Calp. Combo**: Sealed Cartridge, Extraction Buffer Tubes and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the sample collection tube.
- Leave 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 the 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 which is provided with ichroma™-50.
5) Insert test cartridges into the cartridge magazine and insert the cartridge inserted cartridge magazine into the magazine station of ichroma™-50.
6) 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.)
7) ichroma™-50 performs the tests automatically.
8) ichroma™-50 will display the test results 10 minutes after loading samples.

▶ **Instrument: ichroma™ Ⅱ**

1) Collect sample according to the sample collection method using a sampling stick as described 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 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 will cause misleading 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.
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 hemoglobin and calprotectin concentration of the test sample.

Item	iFOB	Calprotectin
Term	ng/mL	mg/kg
Cut off	50 ng/mL (10 μg Hb/g Stool)	50 mg/kg
Working range	25-1000 ng/mL	10-1000 mg/kg
Borderline area, to be repeated (within 4-6weeks)	-	50-100 mg/kg

- The cut-off(reference value) may depend on the test method and the test object. It is recommended to set a cut-off(reference value) for each laboratory.
- In case of a positive result (above 50 ng/ml for iFOB, 50 mg/kg for calprotectin), consult a physician to discuss the test result. The physician may decide further course of action.

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 arises any issues concerning the validity of the test results.
- Control materials are not provided with **ichroma™ iFOB / Calp Combo**. 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**

Item	iFOB (ng/ml)	Calprotectin (mg/kg)
Limit of Blank (LoB)	0.973	1.028
Limit of Detection (LoD)	1.431	1.525
Limit of Quantitation (LoQ)	25	10

- **Analytical specificity**
  - Cross reactivity
    There was no significant cross-reactivity from these materials with the **ichroma™ iFOB / Calp. Combo** test measurements.

Cross-reactivity material	Concentration
Bovine hemoglobin	2000 μg/mL
Chicken hemoglobin	500 μg/mL
Fish hemoglobin	100 μg/mL
Horse hemoglobin	500 μg/mL
Goat hemoglobin	500 μg/mL
Pig hemoglobin	500 μg/mL
Rabbit hemoglobin	500 μg/mL
Sheep hemoglobin	500 μg/mL
Helicobacter pylori	1.2×10^8 CFU/mL
Campylobacter jejuni	1.2×10^8 CFU/mL
Candida albicans	1.2×10^8 CFU/mL
Enterobacter cloacae	1.2×10^8 CFU/mL
Escherichia coli	1.2×10^8 CFU/mL
Pseudomonas aeruginosa	1.2×10^8 CFU/mL

  - Interference
    There was no significant interference from these materials with the **ichroma™ iFOB / Calp. Combo** test measurements.

Interference materials	Concentration
L-ascorbic acid	30 μg/mL
Billirubin	200 μg/mL
Albumin	60 mg/mL
Myoglobin	2 mg/mL
Glucose	120 mg/dL
Triglyceride mixture	500 mg/dL

- **Precision**
  - Between Lot
    One person tested three different lots of **ichroma™ iFOB / Calp. Combo**, five times at each concentration of the control standard.
  - Between person
    Three different persons tested one lot of **ichroma™ iFOB / Calp. Combo**, five times at each concentration of the control standard.
  - Between day
    One person tested one lot of **ichroma™ iFOB / Calp. Combo** during five days; five times at each concentration of the control standard
  - Between site
    One person tested one lot of **ichroma™ iFOB / Calp. Combo** at three different sites, five times at each concentration of the control standard.

Hb	Between-lot		Between-person		Between-day		Between-site	
(ng/mL)	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)
25	24.67	7.61	24.77	8.65	24.55	7.09	24.81	5.97
50	50.00	4.37	49.71	4.37	49.76	4.29	50.46	4.25
250	251.44	2.44	250.83	2.60	251.10	2.35	249.99	2.68

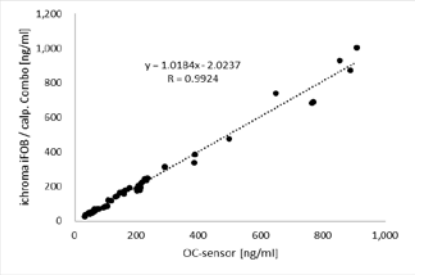
Calp.	Between-lot		Between-person		Between-day		Between-site	
(mg/kg)	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)
10	10.15	3.04	9.78	2.88	10.21	2.53	9.81	3.18
50	49.74	8.86	49.95	7.08	50.91	6.32	50.53	8.4
100	105.34	7.24	102.24	6.42	100.11	7.55	101.52	6.5

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

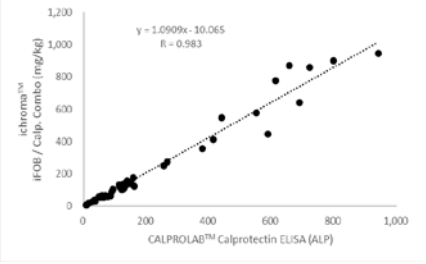
Hb (ng/mL)	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
25	25.17	24.44	24.80	24.80	99%
50	50.69	50.09	49.12	49.97	100%
250	248.57	249.56	251.93	250.02	100%

Calp. (mg/kg)	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
10	10.36	9.39	9.82	9.85	99%
50	50.66	49.45	50.33	50.14	100%
100	99.98	100.13	101.82	100.64	101%

- **Comparability:**
  Hemoglobin concentrations of 50 feces samples were quantified independently with **ichroma™ iFOB / Calp. Combo** and OC-Auto 3 Latex reagent (OC-sensor) 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= 1.0184X – 2.0237 and R = 0.9924 respectively.



Calprotectin concentration of 50 feces samples were quantified independently with **ichroma™ iFOB / Calp. Combo** and CALPROLAB™ Calprotectin ELISA (ALP) as per prescribed test procedures. Test results were compared and their comparability was investigated with linear repression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=1.0909x - 10.065 and R=0.983 respectively.



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

Σ	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