



# AFIAS Calprotectin

### INTENDED USE

**AFIAS Calprotectin** is a fluorescence immunoassay (FIA) for the 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 in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized streptavidin on a test strip. More antigens in the sample will form more antigen antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for AFIAS tests to show calprotectin concentration in the sample.

### COMPONENTS

- AFIAS Calprotectin** consists of 'cartridges' and 'extraction buffer tubes'.
- Each sealed aluminum pouch contains two cartridges.
  - Each cartridge packaged in an aluminum pouch has a component, a cartridge part.
  - The cartridge part contains the membrane called a test strip, which has anti-calprotectin conjugate at the test line, and rabbit IgG at the control line.
  - The extraction buffer contains sodium azide in Tris as a preservative. It is pre-dispensed in extraction buffer tubes. The extraction buffer tubes are 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, extraction buffer tube 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 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 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.
- If test components and/or sample are stored in refrigerator, then allow cartridge to be at room temperature for approximately 30 minutes before use.
- The instrument for AFIAS tests may generate slight vibration during use.
- Used cartridges, extraction buffer tube and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The extraction buffer tube contains sodium azide (NaN<sub>3</sub>), 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.
- AFIAS Calprotectin** will provide accurate and reliable results subject to the below conditions.
  - AFIAS Calprotectin** should be used only in conjunction with the instrument for AFIAS tests.

### LIMITATIONS 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 the 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	Unopened
		1 month	Resealed
Extraction buffer tube	2 - 30°C	20 months	Disposable

- Return an unused cartridge to the spare cartridge zipper bag containing the desiccant pack. Reseal along entire edge of zip-seal.

### MATERIALS SUPPLIED

**REF** SMFP-149  
Components of **AFIAS Calprotectin**

- Cartridge box:
  - Cartridge 24
  - Extraction buffer tube 24
  - Pipette tip (zipper bag) 24
  - Spare cartridge zipper bag 1
  - ID chip 1
  - Instructions for use 1

### MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **AFIAS Calprotectin**.

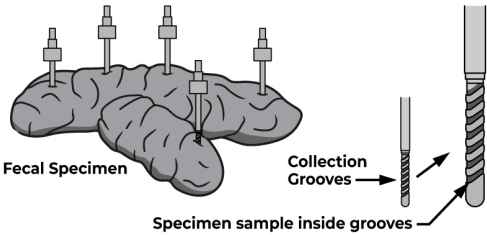
Please contact our sales division for more information.

- Instrument for AFIAS tests**
  - AFIAS-1** **REF** FPRR019
  - AFIAS-3** **REF** FPRR040
  - AFIAS-6** **REF** FPRR020
  - AFIAS-10** **REF** FPRR038
  - Boditech Calprotectin Control** **REF** CFPO-211
  - Boditech Calprotectin Calibrator** **REF** CFPO-380

### SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS Calprotectin** is human feces.

- Collect the sample feces in a clean and dry container.
- Invert an extraction buffer tube and loosen the cap where the sampling stick (yellow color) is attached.
- Poke the sampling stick into the fecal sample about 5 to 6 times at different sites. Whilst collecting the sample with the sampling stick, make sure to exclude large solid lumps. (In case the fecal matter is in liquid form or when using control, transfer 10 µL of the sample to an extraction buffer tube using a pipette.)



- Return the stick to the extraction buffer tube. Tighten the cap thoroughly and shake the tube vigorously around 10 - 15 times so as to disperse the specimen throughout the extraction buffer in the tube.
- Sample (feces) storage periods are as below:
  - Sample (feces) stored at room temperature showed no performance difference for 4 hours.
  - Sample (feces) stored at refrigerator (2~8°C) showed no

performance difference for 72 hours.

- Sample (feces) stored at freezer (-20°C) showed no performance difference for 8 weeks.
- The sample mixture storage periods in extraction buffer tube are as below:
  - The sample mixture in an extraction buffer tube stored at room temperature showed no performance difference for 7 days.
  - The sample mixture in an extraction buffer tube stored at refrigerator (2~8°C) showed no performance difference for 10 days.
- However, it is recommended to use the sample mixture in the extraction buffer on the same day after sampling.
- The storage period may vary depending on the condition and type of feces.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen sample.

### TEST SETUP

- Check the contents of **AFIAS Calprotectin**: Cartridges, extraction buffer tubes, pipette tips, an ID chip, a spare cartridge zipper bag and an Instructions for use.
- Ensure that the lot number of the cartridge matches that of the extraction buffer tube as well as an ID chip.
- If the sealed cartridge and the extraction buffer tube 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 AFIAS tests.
- Insert the ID chip into the 'ID chip port'.
  - ※ Please refer to the instrument for AFIAS tests operation manual for complete information and operation instructions.

### TEST PROCEDURE

#### ► AFIAS-1, AFIAS-3, AFIAS-6

##### General mode

- Collect sample using a sampling stick according to the sample collection method described in the 'sample collection and processing'.
- Hold the tube upside down and break off the black tip on the outside of the black cap.
- Discard 5 drops of reagent onto the paper towel before applying to the cartridge.
- Apply 8 drops of the sample mixture into the sample well of the cartridge.
- Select the 'General mode' in the instrument for AFIAS tests.
- Insert a cartridge into the cartridge holder.
- Insert a tip into the tip hole of the cartridge.
- Tap the 'Start' button on the screen.
- The test result will be displayed on the screen after 10 minutes.

#### ► AFIAS-10

For AFIAS-10, AFIAS Calprotectin can only be used in Emergency mode.

##### Emergency mode – General tip

- Collect sample using a sampling stick according to the sample collection method described in the 'sample collection and processing'.

- 2) Hold the tube upside down and break off the black tip on the outside of the black cap.
- 3) Discard 5 drops of reagent onto the paper towel before applying to the cartridge.
- 4) Apply 8 drops of the sample mixture into the sample well of the cartridge.
- 5) Insert a cartridge into the cartridge holder.
- 6) Insert a tip into the tip hole of the cartridge.
- 7) Tap the ‘load’ button of the bay that holds the cartridge with the tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
- 8) Convert the ‘Emergency mode’ in AFIAS-10.
- 9) Check the tip type (general tip) on the screen.
- 10) Check the sample type (feces) on the screen.
- 11) Tap the ‘Start’ button on the screen.
- 12) The test result will be displayed on the screen after 10 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays calprotectin concentration of the test sample in terms of mg/kg.

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

Working range: 10-1,000 mg/kg

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 **AFIAS Calprotectin**. 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)

- Limit of Detection (LoD)

- Limit of Quantitation (LoQ)

2.82 mg/kg

4.59 mg/kg

10 mg/kg
- 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 feces. **AFIAS Calprotectin** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Concentration
Helicobacter pylori	1.2 x 10 <sup>8</sup> CFU/mL
Campylobacter jejuni	1.2 x 10 <sup>8</sup> CFU/mL
Candida albicans	1.2 x 10 <sup>8</sup> CFU/mL
Enterobacter cloacae	1.2 x 10 <sup>8</sup> CFU/mL
Escherichia coli	1.2 x 10 <sup>8</sup> CFU/mL

Pseudomonas aeruginosa	1.2 x 10 <sup>8</sup> CFU/mL
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- Interference

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

Cross-reactants	Concentration
Human hemoglobin	10 g/dL
Transferrin	400 mg/dL
Prednisolone	8.31 μmol/L
Ciprofloxacin	36.2 μmol/L
Stearic acid	0.4 mmol/L
Palmitic acid	6 mmol/L
Metronidazole	719 μmol/L
Vancomycin	82.8 μmol/L
DMF	2%
DMSO	2%

- Precision**

**Single-site study**

Repeatability (within-run precision)

within-laboratory precision (Total precision)

Lot to lot precision

3 Lots of AFIAS Calprotectin were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
Calprotectin [mg/kg]	Repeatability		Within-laboratory precision		Lot to Lot precision	
	AVG	CV	AVG	CV	AVG	CV
	[mg/kg]	(%)	[mg/kg]	(%)	[mg/kg]	(%)
25	24.07	7.3	24.15	7.0	24.10	7.3
50	47.64	5.6	47.94	5.4	48.64	6.5
250	257.72	4.7	259.94	4.9	259.84	5.2

- Multi-site study**

Reproducibility

1 Lot of AFIAS Calprotectin was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Multi-site study		
Calprotectin [mg/kg]	Reproducibility	
	AVG [mg/kg]	CV (%)
25	24.57	6.9
50	48.19	5.3
250	258.80	4.5

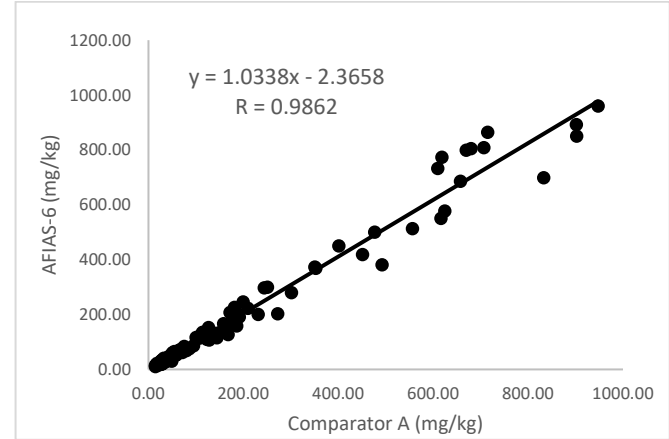
- Accuracy**

The accuracy was confirmed by testing with 3 different lots of AFIAS Calprotectin. The tests were repeated 10 times at each concentration of the control standard.

Calprotectin [mg/kg]	Lot 1	Lot 2	Lot 3	AVG [mg/kg]	Recovery (%)
250.0	259.15	264.24	263.13	262.17	105
205.0	197.36	198.09	197.07	197.50	96
160.0	163.18	170.06	155.69	162.98	102
115.0	116.07	117.60	115.58	116.42	101
70.0	67.02	64.93	67.23	66.39	95
25.0	24.04	24.11	24.57	24.24	97

Comparability

Calprotectin concentrations of 100 clinical samples were quantified independently with AFIAS Calprotectin (AFIAS-6) 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 below.



REFERENCES

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3. Quail, M.A. et al. Fecal Calprotectin Complements Routine Laboratory Investigations in Diagnosing Childhood Inflammatory Bowel Disease. Inflamm Bowel Dis, Vol 15 No 5; May 2009, p. 756-759.
4. Angriman I. et. al. Enzymes in feces: Useful markers of chronic inflammatory bowel disease. Clinica Chimica Acta 381 Feb 2007, p. 63-68.
5. Henderson P, Anderson NH, Wilson DC. The diagnostic accuracy of fecal calprotectin during the investigation of suspected pediatric inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol 2014; 109:637- 645.
6. Walsham NE and Sherwood RA, Fecal calprotectin in inflammatory bowel disease, Clinical and Experimental Gastroenterology, 2016; 9: 21-29.
7. Bjarnason I, The use of fecal calprotectin in inflammatory bowel disease, Gastroenterology & Hepatology, 2017; 13(1): 53-56.

**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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