

# Calprotectin FIA Rapid Test Cassette

Catalogue No.: FIA-CAL-001

*For professional in vitro diagnostic use only.*

## INTENDED USE

The Biopanda Calprotectin FIA Rapid Test Cassette is intended for *in vitro* quantitative detection of Calprotectin in human faeces. Measurement of Calprotectin is used as an aid to assessment of intestinal inflammation.

## SUMMARY

Calprotectin is a 24 kDa dimer of calcium binding proteins S100A8 and S100A9.<sup>1</sup> The complex accounts for up to 60% of the soluble protein content of the neutrophil cytosol.<sup>2</sup> Calprotectin becomes available in the intestinal lumen via leukocyte shedding, active secretion, cell disturbance, and cell death.<sup>3</sup> This results in elevated faecal calprotectin levels, which can be detected in the stool.<sup>3</sup> Elevated faecal calprotectin levels therefore indicate migration of neutrophils into the intestinal mucosa, which occurs during intestinal inflammation.<sup>4</sup> Faecal calprotectin has been used to detect intestinal inflammation, and can serve as a marker for inflammatory bowel diseases.<sup>5</sup> Calprotectin is useful as a marker, as it is resistant to enzymatic degradation, and can be easily measured in feces.<sup>6</sup>

## PRINCIPLE

The Biopanda Calprotectin FIA Rapid Test Cassette detects Calprotectin based on Fluorescence Immunoassay. The sample moves through the strip from sample pad to absorbent pad. The Calprotectin in the sample will be combined with the anti-Calprotectin antibody-1 that has been conjugated with fluorescence microspheres and the combination will be captured by anti-Calprotectin antibody-2 coated on the membrane. The more Calprotectin in the sample, the more fluorescent microspheres will be captured on the membrane. The concentration of Calprotectin in the sample is inversely related to the intensity of the fluorescent signal captured on the T line. According to the fluorescence intensity of the test and the standard curve, the concentration of Calprotectin in the sample can be calculated and reported by the Fluorescence Immunoassay Analyser.

## REAGENTS

The test includes anti-Calprotectin antibody-1 coated fluorescent microspheres and anti-Calprotectin antibody-2 coated on the membrane.

## PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse.
3. Avoid cross-contamination of samples by using a new sample collection container for each sample obtained.
4. This test contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing usual safety precautions (e.g., do not ingest or inhale).
5. Do not eat, drink or smoke in the area where the samples and tests are handled. Handle all samples as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for proper disposal of samples. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when samples are assayed.
6. Do not interchange or mix reagents or ID cards from different lots.
7. Humidity and temperature can adversely affect results.
8. Used testing materials should be discarded in accordance with local regulations.
9. Read the entire procedure carefully prior to any testing.
10. The Biopanda Calprotectin FIA Rapid Test Cassette should only be used with the Biopanda Fluorescence Immunoassay Analyser by medical professionals.

## STORAGE AND STABILITY

1. The kit should be stored at 4-30°C until the expiry date printed on the sealed pouch.
2. The test must remain in the sealed pouch until use.

## 3. Do not freeze.

4. Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

## KIT COMPONENTS

### Materials Provided:

- 10 x foil wrapped Calprotectin test cassettes
- 10 x Sample collection tubes with buffer solution
- 1 x ID card (CAL)
- Package Insert

### Materials required but not provided:

- Timer
- Pipette
- Biopanda Fluorescence Immunoassay Analyser

## SAMPLE COLLECTION AND PREPARATION

### Collection:

Collect sufficient quantity of faeces (1-2 mL or 1-2 g) in a clean, dry sample collection container to obtain maximum antigens (if present).

### Storage and Shipping:

For optimal results, perform the assay within 6 hours after collection. Samples collected may be stored for 3 days at 2-8°C if not tested within 6 hours. For long term storage, samples should be kept below -20°C. If samples are to be shipped, these should be packed in compliance with local regulations covering the transportation of etiological agents.

### Preparation:

Before performing the test, please balance the sample to room temperature (15-30°C). Frozen samples must be completely thawed and mixed well prior to testing.

## DIRECTIONS FOR USE

Refer to the Biopanda Fluorescence Immunoassay Analyser User Manual for the complete instructions on use of the analyser. The test should be conducted at room temperature.

*Allow the test, sample, buffer and/or controls to reach room temperature (15-30°C) prior to testing.*

1. Turn on the Analyser.
2. Take out the ID card and insert it into the ID Card Slot. Choose test mode and/or sample type according to needs.
3. To process faecal samples:
  - **For Solid Samples:**  
Unscrew the cap of the sample collection tube, then randomly stab the sample collection applicator into the faecal sample in **at least 3 different sites** to collect approximately 50 mg of faeces (equivalent to 1/4 of a pea). **Do not scoop the faecal sample.**
  - **For Liquid Samples:**  
Pipette 80 µL of specimen into the specimen collection tube with buffer.
4. Tighten the cap onto the sample collection tube, and then shake the sample collection tube vigorously to mix the sample and the extraction buffer. **Let the tube sit for 2 minutes.**
5. Remove the test cassette from the sealed foil pouch and place it on a clean, level surface. The cassette should be used within one hour of opening. For optimal results, perform test immediately after opening.
6. Hold the sample collection tube upright and open the cap onto the sample collection tube. Invert the sample collection tube and transfer **2 full drops of the extracted sample (approximately 80 µL)** to the sample well of the test cassette, and then start the timer. Avoid trapping air bubbles in the sample well.
7. Test results should be interpreted at **15 minutes** with the use of the Biopanda Fluorescence Immunoassay Analyser.

**Note:** There are different test modes for the Biopanda Fluorescence Immunoassay. The difference between them is incubation of the test cassette is outside or inside the analyser.

Choose test mode accordingly and confirm sample type. Consult the user manual of the analyser for detailed operation information.

Operator must consult the Biopanda Fluorescence Immunoassay Analyser User Manual prior to use and become familiar with the processes and quality control procedures.

## INTERPRETATION OF RESULTS

The result of the Calprotectin test is calculated by the Biopanda Fluorescence Immunoassay Analyser and displayed on the analyser screen. For additional information, please refer to the user manual.

Assay range of Biopanda Calprotectin FIA Rapid Test Cassette is 1-1000 µg/g.

## QUALITY CONTROL

Each Calprotectin FIA Rapid Test Cassette contains an internal control that satisfies routine quality control requirements. This internal control is performed each time a patient sample is tested. This control indicates that the test device was inserted and read properly by the Biopanda Fluorescence Immunoassay Analyser. An invalid result from the internal control causes an error message on the analyser. Insufficient sample volume or incorrect procedural techniques are the most likely reasons for control failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

## LIMITATIONS OF THE TEST

1. The Biopanda Calprotectin FIA Rapid Test Cassette should be used only with the analyser.
2. The Biopanda Calprotectin FIA Rapid Test Cassette is for professional *in vitro* diagnostic use, and should only be used for the quantitative detection of Calprotectin. This test should not be used as the sole criteria for a clinical diagnosis. If the result is abnormal, other clinical findings and alternative test methods are recommended to reach proper medical treatments.
3. The test may yield low results due to Calprotectin epitopes being covered by some unknown components. Low results may also be obtained due to instability or degradation of Calprotectin antigen with time and temperature.
4. Other factors interfering with the test and causing erroneous results include technical/procedural errors, degradation of the test components as well as presence of interfering substances in the test samples.
5. As with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

## EXPECTED RESULTS

Concentrations	Clinical Reference
< 20 µg/g	Negative
≥ 20 µg/g	Positive

Each laboratory should determine the applicability of the reference range through experiments, and establish its own reference value range if necessary to ensure that it can correctly reflect the situation of a particular population.

## PERFORMANCE CHARACTERISTICS

### 1. Method Comparison

For 96 samples, the test results of the Biopanda Calprotectin FIA Rapid Test Cassettes were consistent with commercial Calprotectin test kits and the correlation coefficient ( $R^2$ ) is 0.942.

### 2. Accuracy

The test deviation is  $\leq \pm 15\%$ .

### 3. Assay range

1-1000 µg/g

### 4. Precision

#### Intra-lot precision

Within-run precision has been determined by using 10 replicates of 2 different concentration Calprotectin control. C.V. is  $\leq 15\%$ .

#### Inter-lot precision

Between-run precision has been determined by using 10 replicates for each of three lots using 2 different concentration of Calprotectin control. C.V. is  $\leq 15\%$ .

## REFERENCES

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3. Lehmann, F. S., Burri, E., & Beglinger, C. (2015). The role and utility of faecal markers in inflammatory bowel disease. *Therapeutic advances in*

*gastroenterology*, 8(1), 23-36.

4. Gupta, R. C. (Ed.). (2014). Biomarkers in toxicology. *Academic press*, 272-273.
5. Marshall, W. J., Lapsley, M., Day, A., & Ayling, R. (2014). Clinical biochemistry: metabolic and clinical aspects (3<sup>rd</sup> ed.). *Elsevier Health Sciences*.
6. Tibble, J., Teahon, K., Thjodleifsson, B., Roseth, A., Sigthorsson, G., Bridger, S., ... & Bjarnason, I. (2000). A simple method for assessing intestinal inflammation in Crohn's disease. *Gut*, 47(4), 506-513.

## SYMBOLS USED

The following symbols are used on the packaging and labelling. They are presented here along with their meaning.

	Manufacturer		Expiration date
	Do not re-use test		<i>in vitro</i> diagnostic medical device
	Consult instructions for use		Batch code
	Storage temperature		Contains sufficient for <n> tests
	Catalogue number		Do not use if package is damaged

Thank you for purchasing Biopanda's Calprotectin FIA Rapid Test Cassette. Please read this manual carefully before operating to ensure proper use.



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