

#### INTENDED USE

The Proflow™ Calprotectin test is a single use rapid membrane immunoassay for the qualitative detection of calprotectin in human faecal samples, which may be of use in the diagnosis of inflammatory gastrointestinal disorders. For *In Vitro* Diagnostic Use.

#### SUMMARY AND EXPLANATION

Calprotectin is a calcium-containing protein that makes up 5% of the total protein and 60% of the cytosolic protein of neutrophils. It has bacteriostatic and fungistatic properties and is found in faeces at levels six times higher than that in plasma. This faecal biomarker is useful to assess the activity of inflammatory bowel disease (IBD). IBD includes Crohn's disease (CD) and ulcerative colitis (UC) which are associated with elevated neutrophils.

This faecal calprotectin assay is useful in differentiating IBD from functional irritable bowel syndrome (IBS). It is a simple, non-invasive biomarker that is especially useful in children, who may require general anaesthesia for colonoscopy.

#### PRINCIPLE OF THE TEST

The Proflow™ Calprotectin test is a single use rapid membrane immunoassay for the qualitative detection of calprotectin antigen in human faecal samples.

Monoclonal antibodies to calprotectin antigen are coated onto the test line region of the strip. During testing the sample migrates along the membrane by capillary action and is allowed to react with the conjugate on the test strip. In the case of a positive result the specific antibodies present on the membrane will capture the coloured conjugate.

A green line should always appear in the control line region to show that sufficient volume was added, proper flow was obtained and the reagents functioned correctly. Failure of the control line to appear, whether or not test lines are present, indicates an invalid assay. The test is interpreted by the presence or absence of visibly detectable coloured lines in the test region at 10 minutes or less depending on the concentration of antigen present.

A positive result will show a pink/red test line and a green control line, indicating that calprotectin antigen is present in the sample. A negative test result, read at 10 minutes, will show only a green control line, indicating that calprotectin antigen was not detectable in the sample.

#### MATERIALS PROVIDED

- PL.3130 Proflow™ Calprotectin Test Devices: 20 devices
- PL.3230 Proflow™ Calprotectin Sample Preparation Device: 20 devices
- Package insert

#### MATERIALS REQUIRED BUT NOT PROVIDED

- Sample collection container
- Timer or stopwatch
- Biohazard disposal container
- Disposable gloves

#### STABILITY AND STORAGE

- Store all components at 2-30°C.
- Do not freeze or overheat.
- Do not use beyond the expiration date printed on the outer package label.
- The test kits should be kept away from direct sunlight, moisture and heat.

#### PRECAUTIONS

- For *In Vitro* Diagnostic Use only.
- For professional use only.
- Directions should be read and followed carefully.
- Tests are for single use only. Do not reuse.
- Reagents are provided at the necessary working strength.
- Do not interchange reagents between kits with different lot numbers.
- Do not use kits or reagents beyond the stated expiration dates.
- Microbial contamination of reagents may decrease the accuracy of the assay.
- Treat all materials as if they were infectious and dispose of all material in accordance with local regulation.

#### SAMPLE STORAGE AND COLLECTION

- Collect sufficient quantity of faeces (1-2g or mL for liquid samples).
- Faecal samples should be collected in clean and dry containers (no preservatives or transport media).
- Samples may be stored at 2-8°C for 1-2 days or at -20°C for longer periods before testing.
- Allow all samples to equilibrate to room temperature before testing.
- Freezing and thawing cycles are not recommended.

#### TEST PROCEDURE

Use a separate sample collection vial and test for each sample or control. Allow the tests, faecal samples and buffer to reach room temperature prior to testing.

1. Introduce the swab or stick four times into the faeces up to the thread of the stick to pick up approx. 15mg and put back into the sample preparation device with buffer. For liquid faeces samples, aspirate with a dropper and add 15 µL into the sample preparation device.
2. Shake the sample preparation test to ensure good sample dispersion.
3. Remove the Proflow™ Calprotectin test from its sealed pouch.
4. Break off the top of the vial on the sample preparation device.
5. Dispense 3 drops (100 µL) into the sample well on the test (S).
6. Read the result at 10 minutes.

#### QUALITY CONTROL PROCEDURE

It is recommended that a positive and negative control be used to ensure that the test is performing as expected.

The control line (C) is a procedural control and will show that the test has been performed correctly; proper flow occurred and that the test reagents functioned as expected. When a green line appears at the control line position this indicates the test has been performed correctly. The control line will appear on all valid tests, whether or not the sample is reactive or non-reactive (refer to the Interpretation of Results section).

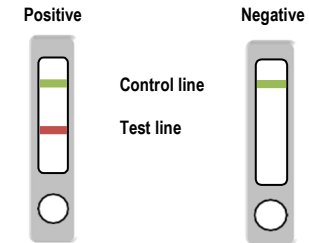
#### INTERPRETATION OF RESULTS

##### Positive

A pink/red line of any intensity appears in the test window at the test line position; a green line will appear at the control line position. This indicates a reactive result that is interpreted as positive for calprotectin.

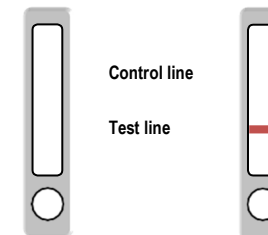
##### Negative

A single green line of any intensity appears in the test window at the control line position. There is no line at the test line position. This indicates a non-reactive result that is interpreted as negative for calprotectin.



##### Invalid

No line appears in the test window at the control line position. This is an invalid result and cannot be interpreted. This is irrespective of whether or not a pink/red line appears in the test window at the test line position. If either condition below occurs, the test should be repeated with a new test.



## LIMITATIONS OF THE PROCEDURE

- The Proflow™ Calprotectin test will only indicate the presence of calprotectin in the sample (qualitative detection) and should be used for the detection of calprotectin in faecal samples only. Neither the quantitative value nor the rate of increase in calprotectin concentration can be determined by this test.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some faecal samples can decrease the intensity of the control line.
- The test must be carried out within 2 hours of opening the sealed bag.
- In the case of patients with active neutrophilic inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, the Proflow™ Calprotectin test would be positive for faecal calprotectin. The Proflow™ Calprotectin test may be used for patients with chronic diarrhoea.
- Faecal samples from patients with non-steroidal anti-inflammatory drug (NSAID) treatment may show a positive result.
- Neonatal faecal calprotectin levels have been reported higher (with an average of 167µg/g) than in normal children.
- Positive results confirm the presence of calprotectin in faecal samples. Nevertheless, it can be due to several causes: inflammatory bowel disease, colorectal cancer and some enteropathies. Positive results should be followed up with additional diagnostic procedures by a clinician to determine the exact cause of inflammation.

## PERFORMANCE CHARACTERISTICS

### EXPECTED VALUES

Higher levels of calprotectin in the stool are associated with an increased risk of relapse in patients with inflammatory bowel disease (IBD). Some studies established equal or higher 50µg hCP/g faeces as a cut-off value to allow detection of adult patients with GI inflammatory problems.

### CUT-OFF VALUE

The cut-off value for the Proflow™ FOB test is 500 ng/ml (50µg hCp/g faeces) for human calprotectin.

### SENSITIVITY AND SPECIFICITY

An evaluation was conducted comparing the results obtained using the Proflow™ Calprotectin test with a commercially available immunoassay test. The Proflow™ Calprotectin test showed:

Specificity >93%  
Sensitivity >94%










### CROSS-REACTIVITY

An evaluation was performed to determine the cross-reactivity of the Proflow™ Calprotectin test. There was no cross-reactivity with common gastrointestinal pathogens, other organisms and substances occasionally present in faeces:

- Haemoglobin (human / bovine / pig)
- Lactoferrin (human / bovine)
- Transferrin (human / bovine / pig)

## REFERENCES

- Bonnin Tomas, A et al. Calprotectina fecal como marcador diferencia entre patologia gastrointestinal orgánica y funcional. *Rev. Esp. de Enf. Dig.* 2007; 99 (12): pp. 689-693.
- Hanai, H. et al. Relationship between Faecal Calprotectin, Intestinal Inflammation, and Peripheral Blood Neutrophils in Patients with Active Ulcerative Colitis. *Digestive Diseases and Sciences*. Sept. 2004; 49 (9): pp 1438-1443.
- Vieira A. et al., Inflammatory bowel disease activity assessed by fecal calprotectin and lactoferrin: correlation with laboratory parameters, clinical, endoscopic and histological indexes. *BMC Research Notes*. 2009; 2:221.

	= Use by
	= Lot number
	= Catalogue number
	= Manufacturer
	= Authorized Representative in the European Community
	= Contains sufficient for <n> tests
	= In vitro diagnostic medical device
	= Temperature limitation
	= Consult instructions for use



Advena Ltd. Tower Business Centre, 2<sup>nd</sup> Floor,  
Tower Street, Swatar, BKR 4013, Malta.

