PathFlow™ Calprotectin Rapid Test Cassette (EN)

MICROGEN BIOPRODUCTS

Instructions for Use

INTENDED USE

The PathFlow™ Calprotectin Rapid Test Cassette (Faeces) is a rapid chromatographic immunoassay for the qualitative detection of Calprotectin in human faeces specimen which might be useful for the diagnosis of inflammatory gastrointestinal disorders.

SUMMARY

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

PRINCIPLE

The PathFlow™ Calprotectin Rapid Test Cassette is a qualitative, lateral flow immunoassay for the detection of Calprotectin in human faeces specimens. The membrane is precoated with anti-Calprotectin antibody on the test line region of the test. During testing, the specimen reacts with the particle coated with Calprotectin antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-Calprotectin antibodies on the membrane and generates a coloured line. The presence of this coloured line in the test line region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a coloured line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test contains anti-Calprotectin antibody particles and anti-Calprotectin antibodies coated on the membrane.

MATERIALS

Provided: Test cassettes, Package insert, Specimen collection tube with extraction buffer.

Not Provided but required: Specimen collection/stool containers, Timer, Droppers.

WARNINGS AND PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- The test cassette should remain in the sealed pouch until use.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test materials should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

- Store as packaged at room temperature or refrigerated (2-30°C).
- The test is stable until the expiration date printed on cassette pouch.
- The test must remain in the sealed pouch until use.
- **DO NOT FREEZE**
- Do not use beyond the expiration date.

SPECIMENS

The stool specimens must be tested as soon as possible after their collection. If necessary, original faces specimen can be stored at 2-8°C for 3 days or -20°C for longer periods of time; extracted specimen in buffer can be stored at 2-8°C for 1 week or -20°C for longer periods of time.

Ensure that the specimen(s) are not treated with cleaning solutions containing formaldehyde or its derivatives.

For optimal results specimen(s) should be tested within 6 hours of collection.

PRECAUTIONS

1. The faecal specimen must be collected in a clean, dry, waterproof container containing no detergents, preservatives or transport media.
2. Bring the necessary reagents to room temperature before use.

DIRECTIONS FOR USE

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

1. **To collect faecal specimens:**

   Collect (1-2mL/1-2g) of faces in a clean, dry specimen collection container to obtain maximum antigens (if present). Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2-8℃ if not tested within 6 hours. For long term storage, specimens should be kept below -20℃.

2. **To process faecal specimens:**

For Solid Specimens:

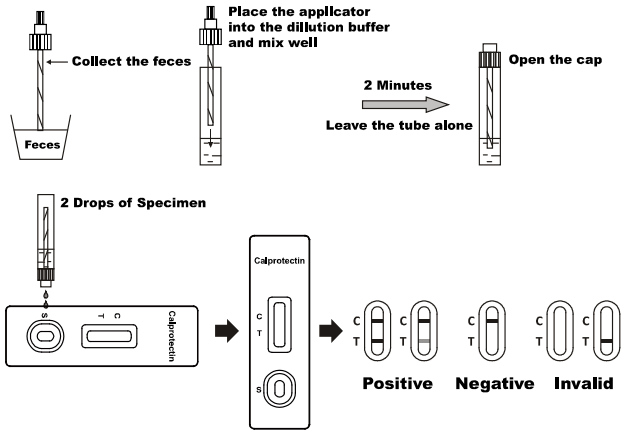
Unscrew the cap of the specimen collection tube , then randomly stab the specimen collection applicator into the faecal specimen in at least 3 different sites to collect approximately 50 mg of faeces (equivalent to 1/4 of a pea). Do not scoop the faecal specimen.

For Liquid Specimens:

Hold the dropper vertically, aspirate faecal specimens, and then transfer 2 drops (approximately 80µL) into the specimen collection tube containing the extraction buffer.

3. Tighten the cap onto the specimen collection tube, then shake the specimen collection tube vigorously to mix the specimen and the extraction buffer. Leave the tube alone for 2 minutes.
4. Bring the pouch to room temperature before opening it. Remove the test cassette from the foil pouch and use it within one hour. Best results will be obtained if the test is performed immediately after opening the foil pouch.
5. Hold the specimen collection tube upright and open the cap of the specimen collection tube. Invert the specimen collection tube and transfer **2 full drops of the extracted specimen** (approximately 80µL) to the specimen well (S) of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.
6. Wait for the coloured line(s) to appear. **The result should be read at 5 minutes.**

Note: *If the specimen does not migrate (presence of particles), centrifuge the extracted specimens contained in the extraction buffer vial. Collect 80µL of supernatant, dispense into the specimen well (S) of a new test cassette and start afresh following the instructions mentioned above.*



INTERPRETATION OF RESULTS

POSITIVE: * Two lines appear. One coloured line should be in the control line region (C) and another apparent coloured line should be in the test line region (T).

***NOTE:** The intensity of the colour in the test line region (T) will vary depending on the concentration of Calprotectin present in the specimen. Therefore, any shade of colour in the test line region (T) should be considered positive.

NEGATIVE: One coloured line appears in the control line region (C). No line appears in the test line region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line 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.

QUALITY CONTROL

Internal procedural controls are included in the test. A coloured line appearing in the control region (C) is an internal valid procedural control. It confirms that sufficient specimen volume has been used and correct procedural technique has been followed.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. The PathFlow™ Calprotectin Rapid Test Cassette is for in vitro diagnostic use only.
2. As with all diagnostic tests, results must be considered with other clinical information available to the physician.

3. Other clinically available tests are required if questionable results are obtained.

EXPECTED VALUE

The Calprotectin Rapid Test Cassette (Faeces) has been compared with another leading commercial rapid test. The correlation between these two systems is 98.5%

LIMIT OF DETECTION/SENSITIVITY

The PathFlow™ Calprotectin Rapid Test Cassette detects Calprotectin at a concentration of 140ng/ml or 50 ng/g or greater.

PERFORMANCE CHARACTERISTICS

The PathFlow™ Calprotectin Rapid Test Cassette has been evaluated with specimens obtained from a population of symptomatic and asymptomatic individuals. Another competitor rapid test served as the reference method – results were found as followed:

Method		**Other Rapid Test**		**Total Result**
PathFlow™ Calprotectin	**Results**	Positive	Negative	
	Positive	133	2	135
	Negative	3	198	201
Total Result		136	200	336

Relative sensitivity: 97.8% (95%CI*: 93.7%~99.5%);

Relative specificity: 99.0% (95%CI*: 96.4%~99.9%);

Accuracy: 98.5% (95%CI*: 96.6%~99.5%).

*Confidence Intervals

INTRA-ASSAY

Within-run precision has been determined by using 15 replicates of three specimens: 140ng/ml, 500ng/ml and 10µg/ml positive specimens. The specimens were correctly identified >99% of the time.

INTER-ASSAY

Between-run precision has been determined by 15 independent assays on the same three specimens: 140ng/ml, 500ng/ml and 10µg/ml positive specimens. Three different lots of the PathFlow™ Calprotectin Rapid Test Cassette have been tested using these specimens. The specimens were correctly identified >99% of the time.

INTERFERING SUBSTANCES

The following analytes were tested as interfering substances: Ascorbic acid 20mg/dl, Oxalic acid 60mg/dl, Bilirubin 100mg/dl, Uric acid 60mg/dl, Aspirin 20mg/dl, Urea 2000mg/dl, Glucose 2000mg/dl, Caffeine 40mg/dl, Albumin 2000mg/dl. No substances showed any interference with the test.

BIBLIOGRAPHY

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TABLE OF SYMBOLS

SYMBOL	DEFINITION
	Batch Code/Lot Number
	In Vitro Diagnostic Medical Device
	Catalogue Reference
	Upper and Lower Temperature Limits
	Expiry Date
	Manufacturer and Address Details
	Date of Manufacture
	Please consult IFU
	Sufficient for
	Do not use if package is damaged
	CE Mark
	Caution

This document applies to the following product codes:
M589CE

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