

Calprotectin and Lactoferrin Combo

Rapid Test Cassette (Faeces)

Package Insert

REF LF/CP	English
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A rapid, one step test for the qualitative detection of Calprotectin and Lactoferrin in human faecal specimen.

For professional *in vitro* diagnostic use only.

[INTENDED USE]

The Calprotectin and Lactoferrin Combo Rapid Test Cassette (Faeces) is a rapid chromatographic immunoassay for the qualitative detection of Calprotectin and lactoferrin in human faecal 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] Lactoferrin is one of the components of the immune system of the body; it has antimicrobial activity (bactericide, fungicide) and is part of the innate defense, mainly at mucosae.^[7] In particular, lactoferrin provides antibacterial activity to human infants.^{[8][9]}

Human lactoferrin, a neutrophil derived glycoprotein, can be measured in faeces and whole gut lavage as an indicator of intestinal inflammation in both IBD and infectious gastroenteritis. Recent studies have shown faecal lactoferrin (FL) as a sensitive biomarker for pediatric IBD. In addition, this biomarker can serve as an aid for guiding the diagnostic and therapeutic process for both pediatric and adult IBD.

[PRINCIPLE]

The Calprotectin Rapid Test Cassette (Faeces) is a qualitative, lateral flow immunoassay for the detection of Calprotectin in human faecal specimen. 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 antibody on the membrane and generate 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.

The Lactoferrin Rapid Test Cassette (Faeces) is a qualitative, lateral flow immunoassay for the detection of lactoferrin in human faecal specimen. The membrane is precoated with anti-lactoferrin antibody on the test line region of the test. During testing, lactoferrin, if present in the specimen reacts with anti-lactoferrin antibody conjugated with coloured particle. The mixture migrates upward on the membrane by capillary action to react with anti-lactoferrin antibody on the membrane and generate 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, anti-Lactoferrin antibody particles, anti-Calprotectin antibody coated on the membrane and anti-lactoferrin antibody coated on the membrane.

[PRECAUTIONS]

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- The test 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 should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

[STORAGE AND STABILITY]

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

[SPECIMEN COLLECTION AND PREPARATION]

- The faecal specimen must be collected in a clean, dry, waterproof container containing no detergents, preservatives or transport media.
- Bring the necessary reagents to room temperature before use.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

[MATERIALS]

Materials Provided

- Test cassettes
- Specimen collection tubes with extraction buffer
- Package insert

Materials Required But Not Provided

- Specimen collection containers
- Timer
- Droppers

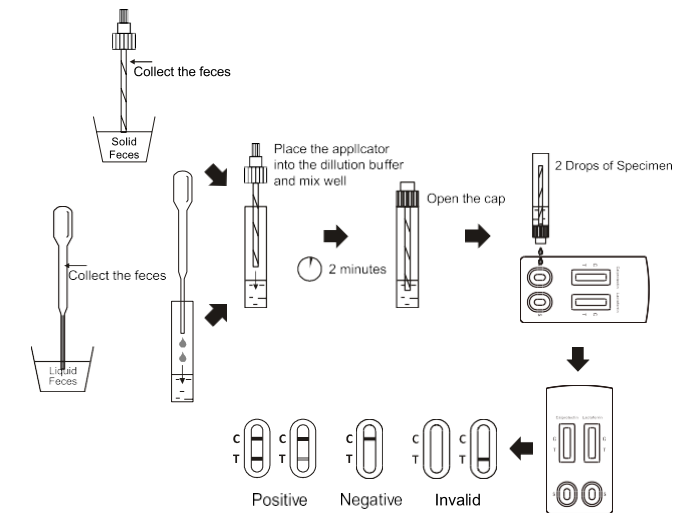
[DIRECTIONS FOR USE]

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

- To collect faecal specimens:
Collect sufficient quantity of faeces (1-2 mL or 1-2 g) 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°C if not tested within 6 hours. For long term storage, specimens should be kept below -20°C.
- 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 80 µL) into the specimen collection tube containing the extraction buffer.
- 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.
- 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.
- Hold the specimen collection tube upright and open the cap onto the specimen collection tube. Invert the specimen collection tube and transfer **2 full drops of the extracted specimen** (approximately 80 µL) to each specimen well of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.
- Read results at 5 minutes after dispensing the specimen.** Do not

read results after 10 minutes.

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



[INTERPRETATION OF RESULTS]

(Please refer to the illustration above)

POSITIVE: Two coloured 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 and Lactoferrin 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 sufficient specimen volume and correct procedural technique. 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]

- The Calprotectin and Lactoferrin Combo Rapid Test Cassette (Faeces) is for *in vitro* diagnostic use only.
- The Calprotectin and Lactoferrin Combo Rapid Test Cassette (Faeces) will only indicate the presence of Calprotectin and Lactoferrin, the detail concentration of Calprotectin and Lactoferrin was not confirmed with the rapid test.
- As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
- Other clinically available tests are required if questionable results are obtained.

The Calprotectin and Lactoferrin Combo Rapid Test Cassette (Faeces) has been compared with another leading commercial Calprotectin rapid test and Lactoferrin rapid test. The correlation between two system is 98.5% for Calprotectin and 98.1% for Lactoferrin.

Detection Limit

Detection limit values of Calprotectin and Lactoferrin Combo Rapid Test Cassette was 50 µg/g or 140ng/ml for Calprotectin, 100ng/ml for Lactoferrin.

Sensitivity - Specificity

The Calprotectin Rapid Test Cassette (Faeces) has been compared with another leading commercial rapid test using clinical specimens.

Method		Other Rapid Test		Total Results
Calprotectin Rapid Test Cassette (Faeces)	Results	Positive	Negative	
	Positive	133	2	135
	Negative	3	198	201
Total Results		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

The Lactoferrin Rapid Test Cassette (Faeces) has been compared with another leading commercial rapid test using clinical specimens.

Method		Other Rapid Test		Total Results
Lactoferrin Rapid Test Cassette (Faeces)	Results	Positive	Negative	
	Positive	41	2	43
	Negative	1	118	119
Total Results		42	120	162

Relative Sensitivity: 97.6% (95%CI*: 87.4%-99.9%)

Relative Specificity: 98.3% (95%CI*: 94.1%-99.8%)

Overall Accuracy: 98.1% (95%CI*: 94.7%-99.6%)

*Confidence Interval

Precision Intra-Assay

Within-run precision has been determined by using 3 replicates of four different specimens containing different concentrations of Calprotectin and Lactoferrin. The specimens were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 3 independent assays on the same four different specimens containing different concentrations of Calprotectin and Lactoferrin. Three different lots of the Calprotectin and Lactoferrin Combo Rapid Test Cassette (Faeces) have been tested using these specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

Cross reactivity with following organisms has been studied at 1.0E+07 organisms/ml. The following organisms were found negative when tested with the Calprotectin and Lactoferrin Combo Rapid Test Cassette (Faeces): *Citrobacter freundii* *Clostridium difficile*

H.Pylori

Candida albicans

Neisseria gonorrhea

Shigella flexneri

Enterococcus faecium

E.coli

Enterococcus faecalis

Gardnerella vaginalis

Proteus mirabilis

Salmonella Infantis

Proteus vulgaris

Pseudomonas aeruginosa

Corynebacterium diphtheria

Interfering Substances

The following compounds have also been tested using the Calprotectin and Lactoferrin Combo Rapid Test Cassette and no interference was observed.

Oxalic acid: 60mg/dl

Aspirin: 20mg/dl

Albumin: 2000mg/dl

Urea: 2000mg/dl

Ascorbic Acid: 20mg/dl

Glucose: 2000mg/dl

Bilirubin: 100mg/dL

Caffeine: 40mg/dl

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Index of Symbols

	Attention, see instructions for use		Tests per kit		Authorized Representative
	For in vitro diagnostic use only		Use by		Do not reuse
	Store between 2-30°C		Lot Number		Catalog #
	Do not use if package is damaged		Manufacturer		Consult Instructions For Use

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