

**Calprotectin and Lactoferrin Combo
Rapid Test Cassette (Feces)**

Package Insert

REF OIBD-625 English

A rapid, one step test for the qualitative detection of Calprotectin and Lactoferrin in human fecal specimen.
For professional *in vitro* diagnostic use only.

INTENDED USE

The Calprotectin and Lactoferrin Combo Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the qualitative detection of Calprotectin and lactoferrin in human fecal 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,^[4] cell disturbance, and cell death.^[5] This results in elevated faecal calprotectin levels, which can be detected in the stool.^[6] Elevated faecal calprotectin levels therefore indicate migration of neutrophils into the intestinal mucosa, which occurs during intestinal inflammation.^[7] Faecal calprotectin has been used to detect intestinal inflammation, and can serve as a marker for inflammatory bowel diseases.^[8] Calprotectin is useful as a marker, as it is resistant to enzymatic degradation, and can be easily measured in faeces.^[9]

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.^[10] In particular, lactoferrin provides antibacterial activity to human infants.^[11]

Human lactoferrin, a neutrophil derived glycoprotein, can be measured in feces and whole gut lavage as an indicator of intestinal inflammation in both IBD and infectious gastroenteritis. Recent studies have shown fecal 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 (Feces) is a qualitative, lateral flow immunoassay for the detection of Calprotectin in human fecal specimen. The membrane is pre-coated 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 colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored 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 (Feces) is a qualitative, lateral flow immunoassay for the detection of lactoferrin in human fecal specimen. The membrane is pre-coated 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 colored particle. The mixture migrates upward on the membrane by capillary action to react with anti-lactoferrin antibody on the membrane and generate a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored 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 fecal 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

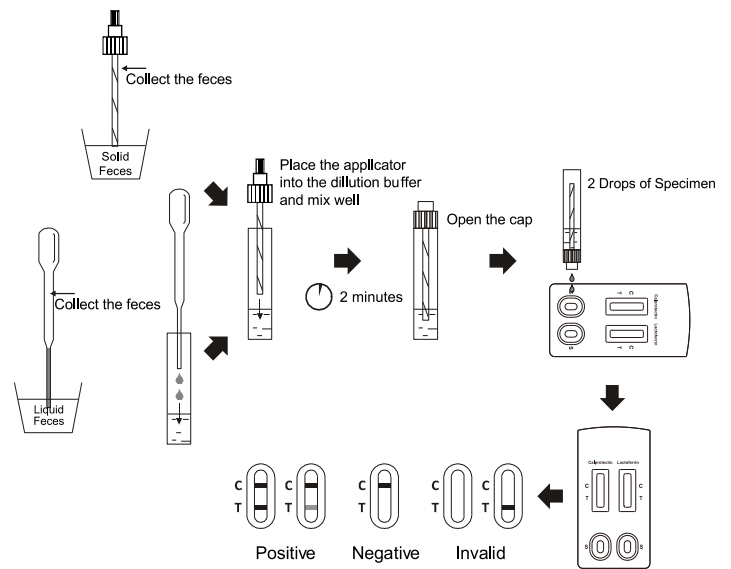
Materials Required But Not Provided

- Specimen collection containers
- Timer
- Droppers

DIRECTIONS FOR USE

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

- To collect fecal specimens:
Collect sufficient quantity of feces (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 fecal specimens:
 - For Solid Specimens:**
Unscrew the cap of the specimen collection tube, then randomly stab the specimen collection applicator into the fecal specimen in at least 3 different sites to collect approximately 50 mg of feces (equivalent to 1/4 of a pea). Do not scoop the fecal specimen.
 - For Liquid Specimens:**
Hold the dropper vertically, aspirate fecal 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 colored lines appear.** One colored line should be in the control line region (C) and another colored line should be in the test line region (T).

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

NEGATIVE: **One colored 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 colored 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 (Feces) is for *in vitro* diagnostic use only.
- The Calprotectin and Lactoferrin Combo Rapid Test Cassette (Feces) 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.

PERFORMANCE CHARACTERISTICS

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 (Feces) has been compared with another leading commercial rapid test using clinical specimens.

Method	Other Rapid Test			Total Results
	Results	Positive	Negative	
	Calprotectin Rapid Test Cassette (Feces)	Positive	133	
	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 (Feces) has been compared with another leading commercial rapid test using clinical specimens.

Method	Other Rapid Test			Total Results
	Results	Positive	Negative	
	Lactoferrin Rapid Test Cassette (Feces)	Positive	41	
	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 (Feces) 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 (Feces):

<i>Citrobacter freundii</i>	<i>Clostridium difficile</i>	<i>H.Pylori</i>
<i>Candida albicans</i>	<i>Neisseria gonorrhoea</i>	<i>Shigella flexneri</i>
<i>Enterococcus faecium</i>	<i>E.coli</i>	<i>Enterococcus faecalis</i>
<i>Gardnerella vaginalis</i>	<i>Proteus mirabilis</i>	<i>Salmonella infantis</i>
<i>Proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>	<i>Corynebacterium diphtheria</i>

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

BIBLIOGRAPHY

1. Brophy, Megan Brunjes; Nolan, Elizabeth M. (16 January 2015). "Manganese and Microbial Pathogenesis: Sequestration by the Mammalian Immune System and Utilization by Microorganisms". ACS Chemical Biology. 10: 150116125412006.
2. Striz, I; Trebichavsky, I (2004). "Calprotectin - a pleiotropic molecule in acute and chronic inflammation." Physiological research / Academia Scientiarum Bohemoslovaca. 53 (3): 245-53.
3. Lehmann, F. S.; Burri, E.; Beglinger, C. (13 October 2014). "The role and utility of faecal markers in inflammatory bowel disease". Therapeutic Advances in Gastroenterology. 8 (1): 23-36.
4. Gupta, Ramesh (2014). Biomarkers in toxicology. San Diego, CA: Academic Press. pp. 272-273. ISBN 9780124046498
5. Marshall, William Marshall.; Lapsley, Marta; Day, Andrew; Ayling, Ruth (2014). Clinical Biochemistry: Metabolic and Clinical Aspects (3 ed.). Elsevier Health Sciences, 2014. ISBN 9780702054785.
6. Tibble J, Teahon K, Thjodleifsson B, Roseth A, Sigthorsson G, Bridger S, Foster R, Sherwood R, Fagerhol M, Bjarnason I (2000). "A simple method for assessing intestinal inflammation in Crohn's disease". Gut. 47 (4): 506-13.
7. Sánchez L, Calvo M, Brock JH (May 1992). "Biological role of lactoferrin". Archives of Disease in Childhood. 67 (5): 657-61.
8. Levin RE, Kalidas S, Gopinadhan P, Pometto A (2006). Food biotechnology. Boca Raton, FL: CRC/Taylor & Francis. p. 1028.
9. Pursel VG (1998). "Modification of Production Traits". In Clark AJ. Animal Breeding: Technology for the 21st Century (Modern Genetics). Boca Raton: CRC. p. 191.

Index of Symbols

	Caution		Tests per kit		Authorized Representative
	For <i>in vitro</i> 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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