



**Calprotectin and FOB Combo
Rapid Test Cassette
(Feces)
Package Insert**

REF TCFC-625 English

A rapid one step test for the qualitative detection of Calprotectin and Human Occult Blood in feces.

For professional in vitro diagnostic use only.

【INTENDED USE】

The Calprotectin and FOB Combo Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the qualitative detection of calprotectin and/or Human Occult Blood in feces.

【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,⁶ 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

Many diseases can cause hidden blood in the feces. This is also known as Fecal Occult Blood (FOB), Human Occult Blood, or Human Hemoglobin. In the early stages, gastrointestinal problems such as colon cancer, ulcers, polyps, colitis, diverticulitis, and fissures may not show any visible symptoms, only occult blood. Traditional guaiac-based methods lack sensitivity and specificity, and also have diet restrictions prior to testing.^{7,8}

【PRINCIPLE】

The Calprotectin and FOB Combo Rapid Test Cassette (Feces) is a qualitative, lateral flow immunoassay for the detection of Calprotectin and Human Occult Blood in feces.

For Calprotectin Rapid Test

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 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.

For FOB Rapid Test

The membrane is precoated with anti-hemoglobin antibody on the test line region of the test. During testing, the specimen reacts with the particle coated with anti-hemoglobin antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-hemoglobin 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, anti-hemoglobin antibody particles and anti-Calprotectin antibody, anti-hemoglobin 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】

For FOB Rapid Test

- Specimens should not be collected during or within three days of a menstrual period, or if the patient suffers from bleeding hemorrhoids or blood in the urine.
 - Alcohol, aspirin and other medications taken in excess may cause gastrointestinal irritation resulting in occult bleeding. Such substances should be discontinued at least 48 hours prior to testing.
 - No dietary restrictions are necessary before using the FOB Rapid Test Cassette.
- For Calprotectin Rapid Test**
- The feces specimen must be collected in clean, dry, waterproof container containing no detergents, preservatives or transport media.
 - Bring the necessary reagents to room temperature before use.

【MATERIALS】

- Materials Provided**
- Test cassettes
 - Specimen collection tubes with extraction buffer
 - Package insert

Materials Required But Not Provided

- Specimen collection containers
- Timer

【DIRECTIONS FOR USE】

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

1. 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.

2. 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.

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 onto the specimen collection tube. Invert the specimen collection tube and transfer **2 full drops of the extracted specimen** (approximately 80 µL) to the each specimen well (S) of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.

6. 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 the specimen well (S) of a new test cassette and start afresh following the instructions mentioned above.

【INTERPRETATION OF RESULTS】

(Please refer to the illustration)

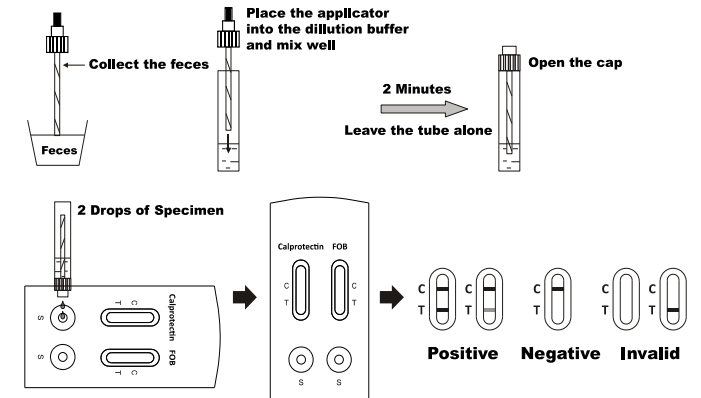
POSITIVE: * **Two colored lines appear.** One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).

***NOTE:** The intensity of the color in the test line region (T) will vary depending on the concentration of Calprotectin and/or FOB 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 FOB Combo Rapid Test Cassette (Feces) is for in vitro diagnostic use only.
- The FOB Rapid Test Cassette will only indicate the presence of Fecal Occult Blood, the presence of blood in feces does not necessarily indicate colorectal bleeding.
- The Calprotectin Rapid Test Cassette will only indicate the presence of Calprotectin, the detail concentration of Calprotectin 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.

【EXPECTED VALUES】

The Calprotectin and FOB Combo Rapid Test Cassette (Feces) has been compared with another leading commercial rapid test. The correlation between these two systems is 98.5% for Calprotectin, 98.6% for FOB.

【PERFORMANCE CHARACTERISTICS】

Accuracy

For Calprotectin Rapid Test Cassette

Calprotectin Rapid Test Cassette (Feces)	Method		Other Rapid Test		Total Result
	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

For FOB Rapid Test Cassette

FOB Rapid Test Cassette (Feces)	Method		Other Rapid Test		Total Result
	Results	Positive	Negative		
	Positive	189	4	193	
Negative	10	802	812		
Total Results			199	806	1005

Relative sensitivity: 189/199=95.0% (95%CI*: 91%–97.6%);

Relative specificity: 802/806=99.5% (95%CI*: 98.7%~99.9%);
 Accuracy: (189+802)/(189+10+4+802) =98.6% (95%CI*: 97.7%~99.2%).
 *Confidence Intervals

Sensitivity

The Calprotectin Rapid Test Cassette can detect levels of Calprotectin as low as 50 µg/g or 140 ng/ml feces.

The FOB Rapid Test Cassette can detect levels of Fecal Occult Blood as low as 50 ng/mL or 6 µg/g feces.

Precision

Intra-Assay

For Calprotectin Rapid Test Cassette:

Within-run precision has been determined by using 3 replicates of these specimens: negative, 140ng/ml, 500ng/ml and 10µg/ml positive specimens. The specimens were correctly identified >99% of the time.

For FOB Rapid Test Cassette:

Within-run precision has been determined by using 3 replicates of these specimens: negative, 50ng/ml, 100ng/ml and 10µg/ml positive specimens. The specimens were correctly identified >99% of the time.

Inter-Assay

For Calprotectin Rapid Test Cassette:

Between-run precision has been determined by 3 independent assays on the same specimens: negative, 140ng/ml, 500ng/ml and 10µg/ml positive specimens. Three different lots of the Calprotectin Rapid Test Cassette have been tested using these specimens. The specimens were correctly identified >99% of the time.

For FOB Rapid Test Cassette:

Between-run precision has been determined by 3 independent assays on the same specimens: negative, 50ng/ml, 100ng/ml and 10µg/ml positive specimens. Three different lots of the FOB Rapid Test Cassette have been tested using these specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

For Caslprotectin:

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 Rapid Test (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>		

For FOB:

The FOB Rapid Test Cassette is specific to human hemoglobin. Specimens containing the following substances were diluted in the extraction buffer to a concentration of 1.0 mg/ml, and tested on both positive and negative controls with no effect on test results:













Bovine hemoglobin	Chicken hemoglobin	Pork hemoglobin,
Goat hemoglobin	Horse hemoglobin	Rabbit hemoglobin
Turkey hemoglobin		

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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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