



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

Positive(+): One color line appears in the control region (C). One color line appears in the test region (T) least.

Test line(T)	Semi quantitative result	Recommended Interpretation
Only T1 appears	Calprotectin level is 50ug/g~200ug/g	Active gastrointestinal inflammation.
T1 and T2 appear	Calprotectin level is >200ug/g	Active gastrointestinal inflammation, risk of relapse in clinical remission, graft rejection or transplantation rejection.

Negative(-): One color line appears in the control region (C). No apparent purple line appears in the test region (T). Negative result showed: There was not Calprotectin in the sample, or the content of Calprotectin below the detectable range.

Test line (T)	Semi quantitative result	Recommended Interpretation
No line	Calprotectin level is below 50ug/g	Neither active gastrointestinal inflammation, nor risk of relapse (CD or UC relapse), nor graft or transplantation rejection

INVALID: C 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 cassette. 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 color line appearing in the control region (C) is internal positive procedural control. It confirms sufficient specimen volume. 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. Calprotectin Semi-Quantitative Cassette (Feces) is for professional in vitro diagnostic use. The test should be used for the detection of calprotectin in human feces specimens only.
2. Calprotectin Semi-Quantitative Cassette (Feces) will only indicate the semi - quantitative level of Calprotectin in the specimen and should not be used as the sole criteria for evaluating inflammatory conditions.
3. Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. Calprotectin values near the cut-off level Test line 1(T1: 50ug/g) and Test line 2 (T2: 200ug/g), should be reported with caution as with all quantitative assays there exists some level of variation. Therefore, a T line with slightly higher intensity than T2 can also represent a value slightly below 200ug/g. Similar observations may occur with values near 50ug/g. A repeat test or further quantitative test is recommended.
5. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended.

EXPECTED VALUES

Calprotectin Semi-Quantitative Cassette (Feces) has been compared with Calprotectin EIA test, demonstrating an overall accuracy of 95%.

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity and Accuracy

Calprotectin Semi-Quantitative Cassette (Feces) has been evaluated with 100 clinical specimens.

Method	Result	ELISA			Total
		0~50ug/g	50~200 ug/g	>200ug/g	
Calprotectin Semi-Quantitative Cassette (Feces)	0~50ug/g	86	0	0	86
	50~200u g/g	2	10	0	12
	>200ug/g	0	0	2	2
Total		88	10	2	100
% Relative Accuracy		97.7%	100%	100%	98.0%
		100%			

Relative sensitivity: 12/12=100% (CI*: 77.9%~100%)
 Relative specificity: 86/88 =97.7% (CI*: 92.0%~99.7%)
 Relative Accuracy: 98/100 =98.0 % (CI*: 93.0%~99.8%)
 *95% Confidence Interval

Precision

Intra-Assay

Assays were carried out to determine assay reproducibility using replicates of 10 tests in three different runs for each of three lots using calprotectin specimen levels at 0 ug/g, 50ug/g and 200ug/g. The specimens were correctly identified >99.9% of the time.

Inter-Assay

Between-run precision has been determined by using the five calprotectin specimen levels at 0 ug/g, 50ug/g and 200ug/g of calprotectin in 3 independent assays. Three different lots of the calprotectin Semi-Quantitative Rapid Test Cassette (feces) have been tested using these specimens. The specimens were correctly identified >99.9% of the time.

Interfering Substances

The following substances do not interfere with the test results at the indicated concentrations: lactoferrin at 3mg/ml, Adenovirus, Rotavirus, E.coli, helicobacter pylori and Salmonella choleraesuis.

BIBLIOGRAPHY

1. Fagerhol MK, Dale I, Andersson T. A radioimmunoassay for a granulocyte protein as a marker in studies on the turnover of such cells. Bull EurPhysiopatholRespir 1980; 16Suppl: 273-282.

Calprotectin Semi-Quantitative Cassette (Feces)

For Professional in vitro diagnostic Use only

For Feces Sample

This package insert is applied to the below products:

A rapid test for the semi-quantitative detection of Calprotectin in feces.

For professional in vitro diagnostic use only.

INTENDED USE

Calprotectin Semi-Quantitative Cassette (Feces) is a rapid chromatographic immunoassay for the semi-quantitative detection of Calprotectin in feces.

SUMMARY

Calprotectin, first described in 1980^[1], is a protein found in the cytosol of neutrophils and macrophages composed of two subunits S100A8 and S100A9. It is released extracellularly in times of cell stress or damage and can be detected within feces and thus can be used as a sensitive marker of intestinal inflammation. It is stable in feces for up to seven days at room temperature and has a homogenous distribution in feces^[2], properties which lend it to testing spot fecal samples.

The inflammatory bowel diseases (IBD)^[3], Crohn's disease^[4] and ulcerative colitis, are chronic relapsing, remitting disorders. Diagnosis along with assessment of disease activity and prognosis present challenges to managing clinicians. Fecal biomarkers, such as fecal calprotectin, are a non-invasive method which can be used to aid the decisions. Fecal protectin has been shown to be useful in the diagnosis of IBD, correlates with bowel mucosal disease activity and can help to predict response to treatment or relapse. With growing evidence supporting its use, over the last decade this fecal biomarker has significantly changed the way IBD is managed^[5].

PRINCIPLE

Calprotectin Semi-Quantitative Cassette (Feces) detects Calprotectin through visual interpretation of color development on the internal strip. Anti-Calprotectin antibodies are immobilized on the test region of the membrane. During testing, the specimen reacts with anti-Calprotectin antibodies conjugated to colored particles and precoated on the sample pad of the test. The mixture then migrates through the membrane by capillary action, and interacts with reagents on the membrane. If no test line appears, it indicates that the Calprotectin level in the specimen is below 50ug/g. If only the test line 1 (T1) appears, it indicates that the Calprotectin level in the specimen is between 50-200ug/g. If all the test lines (T1, T2) appear, it indicates that the Calprotectin level is above 200ug/g. The appearance of control line serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

Calprotectin Semi-Quantitative Cassette (Feces) contains Calprotectin antibody conjugated colloid gold and Calprotectin 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.** Don't use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

1. The feces specimen must be collected in clean, dry, waterproof container containing no detergents, preservatives or transport media.
2. Unscrew the cap of the sample collection device and stick the attached sample collection stick in one go at three different sites into the feces. Only the amount of stool that sticks to the grooves of the sample collection tube
3. Calprotectin Semi-Quantitative Cassette (Feces) is not run within one day of sample collection, the sample collection tube should be stored at 2~8°C, but not longer than 7 days.

MATERIALS

Materials provided

Test cassettes
Sample collection tube with extraction Buffer
Package insert

Materials required but not provided

Specimen collection containers
Timer

TEST PROCEDURE

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

1. To collect fecal specimens:

Collect sufficient quantity of feces (1-2mL or 1-2g) in a clean, dry specimen collection container to obtain enough virus particles. 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:

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 10~50 mg of feces (equivalent to 1/4 of a pea). Do not scoop the fecal specimen.

3. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.

4. Place the cassette on a clean and level surface.

Hold the specimen collection tube upright and unscrew the tip of the specimen collection tube. Invert the specimen collection tube and transfer 2 full drops of the extracted specimen (approximately 80uL) 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.

5. Wait for the colored line(s) to appear. The test result should be read at 10 minutes. Do not interpret the result after 15 minutes.