Calprotectin ELISA

For the quantitative determination of calprotectin in human stool

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 31-CALPHU-E01

Size: 96 determinations

Version: 18.09.2025 / ALPCO 1.1

26-G Keewaydin Drive, Salem, NH 03079 | P: (800) 592-5726 | F: (603) 898-6854 | ts@alpco.com | www.alpco.com

INTENDED USE

The Calprotectin ELISA is intended for the quantitative determination of calprotectin in human stool. For Research Use Only. Not for Use in Diagnostic Procedures.

INTRODUCTION

Human calprotectin is a dimer which consists of the subunits S100A8 (10.835 kDa) and S100A9 (13.242 kDa). The monomers can bind calcium. The complex is in the cytosol of neutrophils and is excreted to the intestine during inflammation (1). The concentration in fecal samples correlates with the severity of inflammatory processes in the intestine. The complex is resistant against enzymatic degradation. The measurement of Calprotectin represents an easy non-invasive analysis of intestinal inflammation.

PRINCIPLE OF THE ASSAY

The Calprotectin ELISA determines human calprotectin according to the "sandwich-principle." Calprotectin in sample, standards, and controls binds to antibodies which are coated to the microtiter plate. After a wash step a peroxidase-labeled detection antibody is added. A second wash step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are read at 450 nm (against the reference wavelength 620 nm) in a microtiter plate reader. The calprotectin concentration can be calculated from the standard curve.

Calibration: The test system is calibrated from a reference preparation of recombinant and purified calprotectin from *E. coli*.

MATERIALS SUPPLIED

31-CALPHU-E01			
Component	**Quantity**	**Preparation**	**Storage**
Microtiter plate, coated	1 plate: 12 x 8-well strips	Ready-to-use	2-8˚C
Universal extraction buffer	150 mL	Ready-to-use	2-8˚C
Wash Buffer concentrate, 10X	100 mL	Dilute 1:10	2-8˚C
Standards*	5 vials, 1.5 mL each	Ready-to-use	2-8˚C
Control 1 and 2*	2 vials, 1.5 mL each	Ready-to-use	2-8˚C
Conjugate: peroxidase-labeled antibody	15 mL	Ready-to-use	2-8˚C
TMB Substrate: tetramethylbenzidine	15 mL	Ready-to-use	2-8˚C
Sample Buffer	120 mL	Ready-to-Use	2-8˚C
Stop solution	10 mL	Ready-to-use	2-8˚C
Plate Sealers	2	Ready-to-use	RT

*Please refer to the Certificate of Analysis enclosed with each kit for Standard and Control concentrations.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge, 3000 x g
- Eppendorf reaction vessels 1.5 ml

- Stool sample extraction vials (80-EXDEV2-100)
- Plastic vials for sample preparation
- Vortex mixer
- Various pipettes
- Multichannel or multipipette
- Foil to cover the microtiter plate (substrate step)
- Distilled or deionized water
- ELISA reader with 450 nm filter (620 nm reference filter)
- Microtiter plate shaker (2 mm orbital shaker capable of 400 rpm)
- Absorbent paper (lint-free)
- Timer

PRECAUTIONS

- This assay is for Research Use Only. Not for use in diagnostic procedures.
- Individual components from different batches and test kits should not be interchanged. The expiry dates stated on the relevant packaging must be observed.
- The test kit reagents contain preservatives to protect against bacterial growth. Avoid contact with the skin and/or mucous membranes.
- The substrate TMB (tetramethylbenzidine) is toxic by ingestion and skin contact. In the event of contact with the skin, the affected area must be washed immediately with plenty of water and soap.
- Avoid contact of the stop solution, which consists of acid, with the skin. It causes burns on contact. Work with protective gloves and goggles. In the event of contact, the burned area must be immediately and thoroughly rinsed with plenty of water. If necessary, a doctor should be consulted.
- Adherence to the test protocol is essential. ALPCO assumes no liability for any damage caused by unauthorized changes in the test procedure.
- The guidelines for carrying out quality control in testing laboratories must be observed. Appropriate controls must be tested.
- The reagents must not be used after the expiration date.
- Wear disposable gloves when handling samples or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or apply makeup in areas where samples or kit reagents are being handled.
- Samples may contain unknown interfering substances. This can lead to false high or false low results.

REAGENT PREPARATION

Microtiter plate - Assemble the required number of strips in the holder. Allow the plate to reach 20-30°C before use. Unused strips must be stored at 2-8°C in the pouch with desiccant. Please do not dispose of the holder until all strips are used.

Wash buffer - Please note: When storing the wash buffer concentrate at 2-8 °C crystallization may occur. Before dilution, all crystals must be dissolved. Dilute the wash buffer concentrate 1:10 with distilled or deionized water (1 part buffer + 9 parts DI water). The 1X working wash

buffer is stable for 14 days at 2-8°C. Dilute only the amount of buffer needed to process the given samples.

All other test reagents are stable at 2-8 °C up to the date of expiry stated on the label, unless otherwise specified.

SAMPLE PREPARATION

Stool samples must be extracted with Universal Extraction Buffer at a ratio of 1:100 (e.g. 10 mg/ml) before testing in the calprotectin ELISA. Manual weighing or an approved stool extraction device (80-EXDEV2-100) may be used.

For manual weighing, mix **15 mg** stool with **1.5 ml** universal extraction buffer (or greater amount of stool diluted **1:100** with universal extraction buffer), then vortex until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge for 10 min at 3000 x g.

The supernatant is diluted **1:38.5** in sample buffer. We recommend **20 µl** supernatant to mix with **750 µl** sample buffer. **100 µl** of the dilution are used in the test per well.

Note: Please use only plastic vials. Do not use glass vials.

ASSAY PROCEDURE

All reagents and samples should be equilibrated to 20-30°C and mixed well before use.
The position of standards, controls, and samples are noted on a protocol sheet.

1. **Wash step**

   Pick out the pre-assembled microtiter plate with the needed number of strips and wash them 1x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate on absorbent paper after the wash step.

2. **Sample incubation**

   Pipette **100 µl Standards, Controls, and diluted samples** in duplicate in the microtiter plate.

   Cover the strips and incubate by shaking for **60 min** at room temperature (20-30 °C; 400 rpm, 2 mm orbit).

3. **Wash step**

   Discard the contents of the microwells and wash 3x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate on absorbent paper after the last wash step.

4. **Conjugate incubation**

   Pipette **100 µl Conjugate** in each microwell.

   Cover the strips and incubate with shaking for **60 min** at room temperature (20-30°C; 400 rpm, 2 mm orbit).

5. **Wash step**

Discard the contents of the microwells and wash 3x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate on absorbent paper after the last wash step.

6. **Substrate incubation**

Pipette **100 µl TMB substrate** in each microwell.

Incubate by shaking for **10-15 min** at room temperature (20-30°C; 400 rpm, 2 mm orbit) in the dark.

7. **Stopping the reaction**

Pipette **50 µl stop solution** in each microwell. Mix well.

8. **Reading**

Read the absorbance at 450 nm. If available, use 620 nm as reference wavelength. Reading should be done within **5 minutes** after stopping the reaction.

CALCULATION OF RESULTS

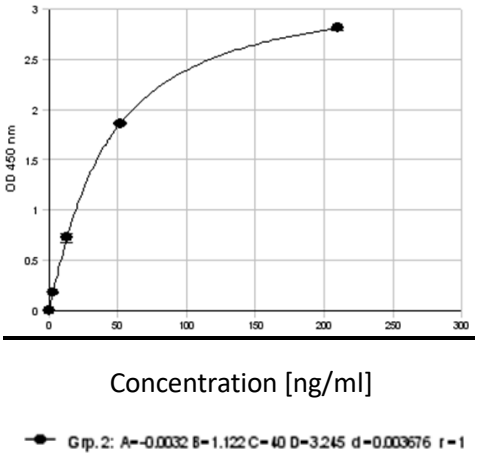
The use of the 4-parameter-Marquardt algorithm is recommended for calculation of results. The calprotectin concentration is multiplied by **3.85.**

Dilution 1: 15 mg in 1.5 ml corresponds to a factor of **100** (assumption: 1 g stool = 1 ml)

Dilution 2: Factor **38.5** (20 µl sample + 750 µl sample buffer)

Calculation: Conc. Sample [µg/ml] = obtained conc. [ng/ml] x 100 x 38.5/1000

TYPICAL STANDARD CURVE



The curve at left is for demonstration purposes only. It must not be used for calculation of sample values.

PERFORMANCE CHARACTERISTICS

Measuring Range
The measuring range of the Calprotectin ELISA is 12.5 - 800 µg/ml.

Sensitivity

*Limit of Detection (LOD): 1.0 ng/ml / *3.85 µg/ml*

For the determination of the detection limit, 20 replicates of Standard 0 were measured. The 3x standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

*The limit of detection is *3.85 µg/ml* following back calculation for sample dilution and with unit conversion.

Limit of Quantification (LOQ): 2.0 ng/ml

For the determination, 20 replicates of Standard 0 were measured. The 10x standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

Precision: Within run (intra-assay) variation

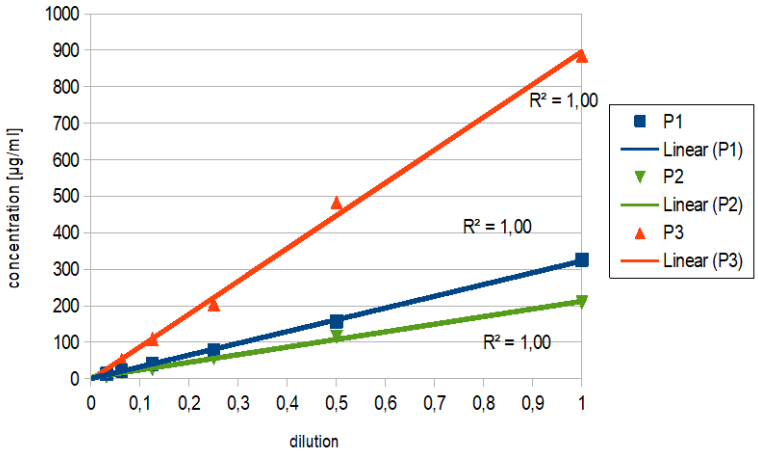
Intra-Assay CV:	4.8 % (20.4 µg/ml)	[n = 10]
	2.9 % (85.1 µg/ml)	[n = 10]
	7.4 % (166 µg/ml)	[n = 10]

Precision: Between run (inter-assay) variation

Inter-Assay CV:	9.1 % (21.7 µg/ml)	[n = 10]
	7.8 % (94.2 µg/ml)	[n = 10]
	4.9 % (166 µg/ml)	[n = 10]

Linearity

Sample dilution was performed with Sample Buffer.



Sample	Dilution	Expected concentration [µg/ml]	Measured concentration [µg/ml]	Recovery [%]
P 1	-		326	-
	1:2	163	157	96.3
	1:4	81.5	78.2	96.0

	1:8	40.8	41.4	101.6
	1:16	20.4	23.2	113.9
	1:32	10.2	13.4	131.4
P 2	-		209	-
	1:2	105	115	110.1
	1:4	52.3	57.2	109.5
	1:8	26.1	27.7	106.0
	1:16	13.1	17.7	135.5
	1:32	6.53	6.54	100.1
P 3	-		884	-
	1:2	442	483	109.3
	1:4	221	205	92.8
	1:8	111	109	98.6
	1:16	55.3	51.8	93.8

Recovery
The recovery was between 88.1 and 103 %.

Sample	Endogenous concentration [µg/ml]	Added concentration [µg/ml]	Expected concentration [µg/ml]	Measured concentration [µg/ml]	Recovery [%]
P 1	21.1	48.8	69.8	61.5	88.1
		195	216	193	89.3
		780	801	754	94.1
P 2	18.2	48.8	67.0	65.2	97.3
		195	213	199	93.3
		780	798	822	103.0
P 3	23.9	48.8	72.6	66.3	91.3
		195	219	210	95.9
		780	804	802	99.8

Cross-Reactivity
Cross reactivity to other proteins could not be detected in stool samples.

Limitations of the Method
Stool samples with calprotectin concentrations above the standard curve should be diluted with sample buffer and measured again. Samples impacted by diarrhea may show normal values.

Disposal
The substrate must be disposed of as a non-halogenated solvent. The stop solution can be neutralized with NaOH and, if the pH value is neutral, it can be disposed of as salt solution (**important:** this reaction produces heat and should be handled carefully). Please refer to local and national guidelines.

REFERENCES

1. Roseth AG, et al. 1999. Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. Scand J Gastroenterol. 34(1): 50-5
2. Sidler MA, et al. 2008. Fecal S100A12 and fecal calprotectin as noninvasive markers for inflammatory bowel disease. Inflamm Bowel Dis. 14(3): 359-66
3. Sipponen T, Kolho KL 2015. Fecal calprotectin in diagnosis and clinical assessment of inflammatory bowel disease. Scand J Gastroenterol. 50(1): 74-80
4. Chen CC, et al. 2012. Fecal calprotectin as a correlative marker in clinical severity of infectious diarrhea and usefulness in evaluating bacterial or viral pathogens in children. J Pediatr Gastroenterol Nutr. 55(5): 541-7
5. Tibble JA, et al. 1999. High prevalence of NSAID enteropathy as shown by a simple faecal test. Gut. 45(3): 362-6
6. Goldstein JL, et al. 2007. Small bowel mucosal injury is reduced in healthy subjects treated with celecoxib compared with ibuprofen plus omeprazole, as assessed by video capsule endoscopy. Aliment Pharmacol Ther. 25(10): 1211-22
7. Rendek Z, et al. 2016. Effect of oral diclofenac intake on faecal calprotectin. Scand J Gastroenterol. 51(1): 28-32
8. Bressler B, et al. 2015. Clinicians´ guide to use of fecal calprotectin to identify and monitor disease activity in inflammatory bowel disease. Can J Gastroenterol Hepatol. 29(7): 369-72
9. Rendek, Z., Falk, M., Grodzinsky, E., Kechagias, S., Hjortswang, H., (2023), Oral omeprazole and diclofenac intake is associated with increased faecal calprotectin levels: a randomised open-label clinical trial, *European Journal of Gastroenterology and Hepathology*, 35(1), 52-58.