

Not for use in the USA

Calprotectin 2 FLUOROENZYMEIMMUNOASSAY FOR CALPROTECTIN DETERMINATION FOR IN VITRO DIAGNOSTIC USE

DIRECTIONS FOR USE

CONTENTS

EliA uses a modular reagent system. All information needed to understand the use of the EliA tests can be found in the analyte specific DfU and the corresponding EliA Control DfU.

INTENDED USE

EliA Calprotectin 2 is intended for the in vitro quantitative measurement of calprotectin in human stool as an aid in the clinical diagnosis of inflammatory bowel diseases (IBD). EliA Calprotectin 2 uses the EliA Calprotectin 2 method on the instrument Phadia 250.

SUMMARY AND EXPLANATION OF THE TEST

Calprotectin is a calcium- and zinc-binding protein which is predominantly present in the cytoplasm of cells involved in pathogen defense, such as neutrophil granulocytes, monocytes and macrophages.^{1,2} In neutrophil granulocytes it accounts for as much as 60% of the cytosolic protein. In intestinal inflammation neutrophil granulocytes migrate through the intestinal wall into the intestinal lumen, which leads to an elevated calprotectin level in the stool.³ The level of fecal calprotectin correlates directly with the number of neutrophil granulocytes in the intestinal lumen and is thus specifically elevated in inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis. Fecal calprotectin levels get affected by nonsteroidal anti-inflammatory drug (NSAID) intake, bleeding more than 100 ml and by malignancy.⁴ Fecal calprotectin measurement is an easy, non-invasive first line test which clearly differentiates IBD from IBS (irritable bowel syndrome) and other functional disorders. It has been shown to be the most sensitive and most specific test for this discrimination, clearly outperforming blood tests such as CRP or ESR.⁵ Fecal calprotectin correlates with disease activity and is able to predict relapses in IBD.⁶ This makes fecal calprotectin useful for both diagnosis and monitoring of IBD patients.

PRINCIPLES OF THE PROCEDURE

The EliA Calprotectin 2 Wells are coated with monoclonal antibodies to calprotectin. If present in the patient's specimen, calprotectin binds to the coated antibodies. After washing away non-bound components, enzyme-labeled antibodies against human calprotectin (EliA Calprotectin 2 Conjugate) are added to form a calprotectin-conjugate complex. After incubation, non-bound conjugate is washed away and the bound complex is incubated with a Development Solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The higher the response value, the more calprotectin is present in the specimen. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

REAGENTS / MATERIAL

The EliA reagents are available as modular packages, each purchased separately. All packages except for the EliA Calprotectin Positive Control 250, the EliA Calprotectin Negative Control 250 and the EliA Stool Extraction Kit 2 are required to carry out an EliA Calprotectin 2 Test.

The EliA Calprotectin 2 Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.

EliA Calprotectin 2 Test-Specific Reagents

EliA Calprotectin 2 Well (Art. No. 14-6748-01)

Calprotectin 2 Well; short name: cn2	coated with monoclonal antibodies to calprotectin	4 carriers (16 wells each); sufficient for 64 determinations	ready for use; store dry at 2-8 °C until expiration date
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EliA Calprotectin Positive Control 250 (Art. No 83-1083-01)

Human calprotectin in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: pos	Control containing human calprotectin	6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial	Ready for use; store at 2-8 °C until expiration date
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EliA Calprotectin Positive Control 250 is prepared from human blood.

EliA Calprotectin Negative Control 250 (Art. No 83-1085-01)

Human calprotectin in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: neg	Control containing human calprotectin	6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial	Ready for use; store at 2-8 °C until expiration date
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EliA Calprotectin Negative Control 250 is prepared from human blood.

EliA Method-Specific Reagents (Phadia 250)

EliA Calprotectin 2 Extraction Buffer (83-1147-01)

Calprotectin 2 Extraction Buffer (red colored); Tris-buffer containing BSA and sodium azide (0.05 %)	6 bottles (117 ml each); sufficient for 6 x 15 extractions	ready for use, shake before using; store at 2-8 °C until expiration date
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EliA Sample Diluent (Art. No 83-1023-01)

Sample Diluent (yellow colored); PBS containing BSA, detergent and sodium azide (0.095 %)	6 bottles (48 ml each); sufficient for ≥ 6 x 180 dilutions	ready for use; store at 2-8 °C until expiration date
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EliA Calprotectin 2 Conjugate 50 (Art. No 83-1127-01)

Calprotectin 2 Conjugate (blue colored); β-Galactosidase anti-calprotectin (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-C2	6 wedge shaped bottles (5 ml each); sufficient for 6 x 50 determinations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE DO NOT REUSE
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EliA Calprotectin 2 Conjugate 200 (Art. No 83-1128-01)

Calprotectin 2 Conjugate (blue colored); β-Galactosidase anti-calprotectin (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-C2	6 wedge shaped bottles (19 ml each); sufficient for 6 x 200 determinations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE DO NOT REUSE
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EliA Calprotectin 2 Calibrator Strips (Art. No 83-1123-01)

Human calprotectin (0, 3, 10, 20, 200, 750 ng/ml); in PBS containing BSA, detergent and sodium azide (0.095 %) symbol: Cal-0, Cal-3, Cal-10, Cal-20, Cal-200, Cal-750	5 strips 6 single-use vials per strip (0.3 ml each); sufficient for one calibration curve (double determination)	ready for use; store at 2-8 °C until expiration date
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Manufactured from human blood

EliA Calprotectin 2 Curve Control Strips (Art. No 83-1129-01)

Human calprotectin (20 ng/ml); in PBS containing BSA, detergent and sodium azide (0.095 %) symbol: CC-1	5 strips Each strip contains 6 x 0.3 ml CC-1 (double determination)	ready for use; store at 2-8 °C until expiration date
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Manufactured from human blood.

EliA Calprotectin 2 Calibrator Well (Art. No 14-5667-01)

Calprotectin 2 Calibrator Well coated with mouse monoclonal antibodies; short name: C2cal	4 carriers (16 wells each); sufficient for 64 determinations	ready for use; store dry at 2-8 °C until expiration date
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EliA Stool Extraction Kit 2 (Art. No 14-5651-01)

Stool extraction tubes pre-filled with 1300 µl of EliA Calprotectin 2 Extraction Buffer	50 tubes; sufficient for 50 stool sample extractions	ready for use; store at 2-8 °C until expiration date
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Phadia 250 General Reagents

Development Solution (Art. No. 10-9440-01)

Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside, <0.0010% preservative*	6 bottles (17 ml each); sufficient for 6 x >170 determinations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE
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Stop Solution (Art. No. 10-9442-01)

Stop Solution 4 % Sodium Carbonate	6 bottles (119 ml each); sufficient for 6 x >560 determinations	ready for use; store at 2-8 °C until expiration date
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Dilution Plates (Art. No. 12-3907-08)

MicroWell™ plates with 96 wells, 0.5 ml each; Polypropylene	100 plates per package; sufficient for 100 x 96 samples	ready for use DO NOT REUSE
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* Preservative: mixture of 5-chloro-2-methyl-2H-isothiazol-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1).

Washing Solution (Art. No. 10-9422-01/10-9202-01)

For information see separate Washing Solution package insert.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use reagents beyond their expiration dates.
- We do not recommend to pool reagents.
- Some of the reagents are manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for anti-

bodies to HIV1, HIV2 and hepatitis C virus and found negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or local and national guidelines on laboratory safety procedures.

WARNING! Reagents contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or absorbed by skin or eyes. NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local and national guidelines. Waste Bottle and ImmunoCAP/EliA Well Waste Container may be contaminated by potentially infectious material. Use appropriate safety measures and wear gloves.

Indication of Instability

Phadia 250 Instrument Software has built-in acceptance limits for the calibration curve and the curve control. EliA Wells are moisture sensitive. An activity loss that might occur due to inappropriate handling can be detected using the appropriate EliA Control. For more information see Phadia 250 User's Guide/Reference Manual.

INSTRUMENT

The Phadia 250 Instrument processes all steps of the test. For further information regarding test set-up, instrumentation and software etc. see Phadia 250 User's Guide/Reference Manual.

SPECIMEN COLLECTION, HANDLING AND PREPARATION

The procedure can be performed with human stool specimens. Avoid repeated freezing and thawing. Samples should be stored in aliquots at -20 °C (-4 °F) or below for repeated measurements.

The stool samples can be extracted by two different methods:

A) Stool extraction using the EliA Stool Extraction Kit 2 (order no. 14-5651-01, 50 tubes). The extraction tubes are pre-filled with 1300 µl of EliA Calprotectin 2 Extraction Buffer.

Extraction procedure:

1. Unscrew the tube's cap by turning the upper, light blue part of cap to the left and pull out the light blue rod.
2. Insert the light blue rod into the stool sample. Be sure that the four notches at the lower part of the rod are completely covered with stool.
3. Insert the rod back into the tube carefully. Excess material will be stripped off, and a defined amount of stool sample will remain in the notches.
4. Lock the tube firmly by turning the light blue part of the cap to the right.
5. Homogenize the stool sample completely using a vortex mixer. Afterwards incubate for 10 minutes. Make sure that no stool sample stays in the notches. In case of very solid stool samples it may help to soak the sample in the tube for 10 min before dissolving it.
6. Unscrew the complete cap by turning the lower, dark blue part of the cap to the left. Discard complete cap and rod.
7. Transfer the homogenate to an Eppendorf tube and centrifuge for 5 minutes at 3000 x g.
8. Transfer the supernatant to a fresh tube.

The supernatant is the raw sample used for testing. The extract can be stored at room temperature for max. 3 days, at 4°C for max.7 days, and at ≤ -20°C for max. 3 months.

B) Stool extraction using conventional stool extraction devices which are not pre-filled with extraction buffer. We recommend the “Fecal sample preparation kit” by Roche Diagnostics for stool extraction. This kit is available through Thermo Fisher Scientific (order no. 14-5619-01, 50 tubes).

Extraction procedure:

1. Weigh the empty extraction device.
2. Transfer approximately 100 mg of homogeneous stool sample to the extraction device.
3. Weigh the transferred amount of stool.
4. Add 75 times the stool weight of EliA Calprotectin 2 Extraction Buffer (e.g. 100 mg stool sample + 7,5 ml buffer).
5. Lock the tube firmly.
6. Homogenize the sample completely using a vortex mixer. Afterwards incubate for 10 minutes.
7. Transfer 1-2 ml of the homogenate to an Eppendorf tube and centrifuge for 5 minutes at 3000 x g.
8. Transfer the supernatant to a fresh tube.

The supernatant is the raw sample used for testing. The extract can be stored at room temperature for max. 3 days, at 2-8°C for max.7 days, and at ≤ -20°C for max. 3 months.

Sample Dilution

Samples must be diluted with EliA Sample Diluent. A 1:200 dilution of the samples is required for the EliA Calprotectin 2 Test. Samples can be diluted manually, but instrument dilution is recommended.

PROCEDURE

Handling of EliA Calprotectin 2 Well

In the Phadia 250 storage chamber, carriers are stable for up to 28 days. If you are not expecting to use them up within this time, the carriers should be loaded via the Phadia 250 Loading Tray and, for stability reasons, must be put back into the desiccant-containing foil bag directly after the run. Because it is important to store the wells in dry conditions at 2-8°C, the bag must be properly resealed. If stored under these conditions, the shelf-life from the date of first opening is 9 months, if not limited by the expiry date stated on the carrier and foil bag.

Lot specific barcode

Use the built-in barcode reader to enter the lot specific information of EliA Calprotectin 2 Well, EliA Calprotectin 2 Calibrator Well and EliA Calprotectin 2 Conjugate. In case of manual handling make sure to enter the characters below the barcode.

On-board stability of reagents

• **EliA Wells**

EliA Well carriers can be stored on-board for 28 days at 2-8°C or 24 hours at room temperature.

• **EliA Calibrator Strips, EliA Curve Control Strips**

Can be stored on-board for 28 days.

• **EliA Sample Diluent**

Can be stored on-board for 7 days at room temperature. Re-cap bottles every night.

• **EliA Conjugate**

Single use reagent, open vials must not be stored.

• **Development Solution**

Can be stored on-board for a total of 40h at room temperature. Can be used 5 times during shelf life and be stored at room temperature for 8 hours on each occasion. Re-cap bottles every night. During weekends or longer interval between instrument usage it is recommended to store bottles at 2-8°C.

• **Stop Solution**

Can be stored on-board for 7 days at room temperature. Re-cap bottles every night.

• **Washing Solution**

Prepared solution can be stored on-board for 7 days at room temperature. Discard every seventh day and perform weekly maintenance according to instrument user manual.

Volumes per determination

Reagent volumes per determination

Calibrator	90 µl
EliA Calprotectin 2 Conjugate	90 µl
Development Solution	90 µl
Stop Solution	200 µl

Sample volumes per determination

Manual dilution:	90 µl of diluted sample
Instrument dilution (1:200):	10 µl of non diluted sample

For tube-specific dead volumes see Phadia 250 User's Guide/Reference Manual.

Reagent volumes per 200 determinations

Washing Solution	5-7 l*
Rinse Solution	5-6 l*

* The residual volume depends on the number of samples and dilution method used.

Procedural comments

- From one sample diluted by the instrument (1:200), up to 11 determinations can be made.
- When using software default, samples are run in single determination.
- Washing Solution must be at room temperature when used.
- The first result is available after approx. 2 hours and further results at one minute intervals afterwards. Up to 5 x 10 samples can be loaded continuously and are processed by random access.
- Incubations are automatically performed at 37 °C (98.6 °F).

CALIBRATION AND REFERENCE MATERIAL

The calibration curve is obtained with EliA Calprotectin 2 Calibrators which are run in duplicate. The curve is stored and subsequent tests are evaluated against the stored curve using only the EliA Calprotectin 2 Curve Control (run in duplicate).

A new calibration curve must be run when:

- the last calibration was made more than one month ago or
- a new lot of EliA Calprotectin 2 Conjugate is introduced or
- when the EliA Calprotectin 2 Curve Control is outside the specified limits (defined in Phadia 250 Instrument Software).

There are no international standards for calprotectin. Results are given in mg/kg.

QUALITY CONTROL

Control Specimens

Good laboratory practice requires that quality control specimens should be included in every run. Any material used should be assayed repeatedly to establish mean values and acceptance ranges. EliA Controls are available from Phadia for the quality control of the measurements.

CALCULATION AND INTERPRETATION OF RESULTS

Presentation of Results

Phadia 250 measures calprotectin concentrations in ng/ml. By using a conversion factor given by the lot-specific code of the EliA Calprotectin 2 Well, the results are automatically converted to mg/kg.

Interpretation of Test Results

The ranges (negative, positive) recommended for the evaluation of the results are given in the table below.

Test	Unit	negative	positive
EliA Calprotectin 2	mg/kg	≤ 50	> 50

Good laboratory practice requires that each laboratory establishes its own range of expected values.

LIMITATIONS

A definitive clinical diagnosis should not be based on the results of a single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

EXPECTED VALUES

Calprotectin is positive in a certain percentage of the normal population, strongly depending on age. While 11 % of children have been reported to be positive⁷, it is 25 % of subjects between 50 and 70 years of age⁸. Expected values may vary depending on the population tested.

Results Obtained for Healthy Subjects

The frequency distribution for calprotectin was investigated in a group of apparently healthy subjects equally distributed by age and gender, using stool samples from a Caucasian population. The results are given in the table below.

Test	Unit	No. of samples	Mean value	95%-percentile	99%-percentile
EliA Calprotectin 2	mg/kg	99	10.7	32.0	47.1

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range (detection limit, upper limit) for EliA Calprotectin 2 is from 3.8 to ≥ 6000 mg/kg. No hook effects could be observed for concentrations up to 14-fold above the measuring ranges.

Only values above the Detection Limit can be regarded as valid results. The upper limit of the reported results can vary due to a lot-specific conversion from ng/ml to mg/kg. Results above the upper limit are reported as “above”.

Please note that not all samples can be diluted linearly within the measuring range.

Specificity

The EliA Calprotectin 2 Test permits the determination of human calprotectin as described in section “Reagents”.

Precision

To determine the precision of the assay, the variability was assessed in studies with 21 runs by examining the samples in 252 replicates on 3 instruments over 7 days with a calibration curve in each run. The statistical evaluation was performed by Analysis of Variance. The results are given in the table below.

Test	Sample	Unit	Mean value	Coefficients of variation (%)	
				Intra-Run	Inter-Run
EliA Calprotectin 2	1	mg/kg	59.1	2.5	2.0
	2	mg/kg	1126	2.4	2.7
	3	mg/kg	4706	2.5	3.3










WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Phadia AB may affect the results, in which event Phadia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Phadia AB and its authorized distributors, in such event, shall not be liable for damages, indirect or consequential.

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 LOT	Batch code		Contains x determinations
	Biological Risk		Read Directions for Use
	Store at 2-8°C/35-46°F		Manufactured by
	Expiration date		Do not reuse in a second run
 IVD	For <i>in vitro</i> diagnostic use		

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Revision History



Version	Countries	Change
21	all, except us	Phadia 250 and Phadia 2500/5000, chapter " SUMMARY AND EXPLANATION OF THE TEST": Insertion of the sentence " Fecal calprotectin levels get affected by nonsteroidal anti-inflammatory drug (NSAID) intake, bleeding more than 100 ml and by malignancy"
	all, except us	Phadia 250 and Phadia 2500/5000, chapter "SPECIMEN COLLECTION, HANDLING AND PREPARATION", at the end of both parts A and B: Incorporation of the stool extract stability
	all, except us	Phadia 250 and Phadia 2500/5000, chapter "LIMITATIONS": Deletion of the sentence "Blood in the stool may affect EliA Calprotectin results"
	all, except us	Phadia 250 and Phadia 2500/5000, chapter "REFERENCES": Insertion of a new literature article (reference number 4) and re-numbering of the following references.

