# CALPROLAB™ Calprotectin ELISA (ALP)

CE

## 1. INTENDED USE

The **CALPROLAB™ Calprotectin ELISA (ALP)** is a quantitative method for the determination of Calprotectin in stool samples and can thus be used as an aid in identifying organic disease of the small intestine, large bowel or the stomach in patients, to determine the disease activity and monitor the response to treatment in patients with ulcerative colitis or Crohn's disease.

The **CALPROLAB™ Calprotectin ELISA (ALP)** has been validated for stool samples.

**The test is for *in vitro* diagnostic use.**

## 2. BACKGROUND

Various types of organic diseases in the gastrointestinal tract may cause damage to the intestinal epithelial lining (mucosa layer). Such damage may vary from increased permeability of the mucosa to inflammation and ulcerations. The bowel content is rich in bacteria and other microorganisms releasing substances which may be toxic or chemotactic, i.e. they stimulate leukocytes, in particular polymorphonuclear neutrophilic granulocytes (PMN), to migrate into the gut lumen where they release their contents including antimicrobial substances like Calprotectin. This protein constitutes about 60% of total proteins in the cytoplasm of PMNs [2)] and can be reliably estimated in faecal samples stored for up to seven days at ambient temperature [3)].

Calprotectin is a 36 kilodalton calcium and zinc-binding protein [4)], produced by PMNs, monocytes and squamous epithelial cells (except those in normal skin) [5,6)]. After binding of calcium, it can resist degradation by leukocytic and microbial enzymes [3,7)]. By competing with different enzymes for limited, local amounts of zinc, Calprotectin can inhibit many zinc-dependent enzymes [8)] and thereby kill microorganisms or animal and human cells in culture [9,10)]. Different types of disease, for instance bacterial infections, rheumatoid arthritis and cancer, lead to activation of PMNs and increased levels of Calprotectin in plasma, cerebrospinal fluid, synovial fluid, crevicular fluid, urine or other human materials [1)].

It is of special importance that the concentration of Calprotectin in faeces is correlated with the number of PMNs migrating into the gut lumen [11)], and that it can be detected reliably even in small (less than one gram) random stool samples [3,12)]. Furthermore, organic diseases of the bowel give a strong Calprotectin signal, i.e. elevations are regularly five to several thousand times the upper reference in healthy individuals [3,13,14,15)], indicating intestinal inflammation.

Inflammatory bowel diseases (IBD), i.e. ulcerative colitis and Crohn's disease, may appear from early childhood to late adulthood and the diagnosis is often delayed due to vague symptoms or reluctance to perform endoscopy and biopsy. The **CALPROLAB™ Calprotectin ELISA (ALP)** can contribute to an earlier diagnosis of IBD since the test is usually positive in active IBD.

Functional disorders like irritable bowel syndrome (IBS) do not give increased faecal Calprotectin concentrations, but organic abdominal disorders like IBD do. Patients with organic and functional abdominal disorders may have similar symptoms, and clinical examination alone may not be sufficient to give a specific diagnosis. Further diagnostic procedures are complex, expensive and may expose the patient to pain and other risks. A test for faecal Calprotectin is a simple, non-invasive, inexpensive and objective method that can help selecting patients for additional examination like endoscopy. Abdominal symptoms are very common both in children and adults and a negative result as measured by the **CALPROLAB™ Calprotectin ELISA (ALP)** can with high probability rule out inflammatory bowel disorders [13)].

Mucosal healing is the optimal goal for IBD treatment, and a test for faecal Calprotectin can tell when this has been achieved. Many IBD patients in clinical remission with normal C-reactive protein (CRP) levels still have on-going inflammation [16)], reflected by increased faecal Calprotectin. Such patients have increased risk of relapse within a few months [17)]. If mucosal healing can be achieved, the risk of


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relapse and need for major abdominal surgery will be reduced [18,19]. Normalisation of Calprotectin levels means that mucosal healing has been achieved [20]. The risk and severity of side effects to treatment should be balanced against the risk of continued inflammation, severe clinical relapse and complications.

The importance of achieving mucosal healing has been the focus of many scientific reviews [21-29] and articles [30-35].

Our Calprotectin ELISA technology has also been used for analysis of serum and plasma samples, and high Calprotectin levels have been found in patients with bacterial infections [36-37], sepsis and inflammatory conditions like rheumatoid arthritis (RA) and Systemic Lupus Erythematosus (SLE) [38-42]. Scientific papers show that Calprotectin in serum/plasma is a superior marker of disease activity in RA and SLE and can predict flares. It is also a good marker of acute coronary artery events leading to myocardial infarction [43-45]. A recommended sample procedure together with some important test specifications for plasma (EDTA) testing is given in chapter 7.2 and 13.

Calprotectin determination has also been done in other body fluids, secretions and excretions, for instance crevicular fluid and urine. The Calprotectin concentrations and protocols vary in the different body fluids and have to be performed according to the published methods (e.g.[1]).

## 3. PRINCIPLE OF THE TEST

The **CALPROLAB™ Calprotectin ELISA (ALP)** is based upon preparation of an extract of faeces using our patented Faecal Extraction Buffer. The level of Calprotectin is determined by testing the extract in an enzyme-linked immunoassay (ELISA) specific for Calprotectin.

In the ELISA, samples and standards are incubated in separate microtiter wells coated with monoclonal antibodies which bind the Calprotectin. After incubation and washing of the wells, bound Calprotectin is allowed to react with enzyme-labelled, immunoaffinity-purified Calprotectin-specific antibodies. After this reaction, the amount of enzyme bound in the microtiter wells is proportional to the amount of Calprotectin in the sample or standard, which is determined by incubation with a substrate for the enzyme giving a coloured product. The colour intensity is determined by absorbance using an ELISA plate reader, and is proportional with the concentration of Calprotectin in the standards and samples. The assay is calibrated using Calprotectin purified from leukocyte extract.

## 4. MATERIALS

### 4.1. Reagents supplied with the kit

- MTP **Coated microtiterplate:** 12 strips, 8 wells per strip, coated with affinity-purified monoclonal mouse antibodies specific for Calprotectin. The plate is stored in a sealed bag with desiccant.
- DIL 5x **Sample Dilution Buffer (5x conc.) ***:** 1 x 20 mL, 5x concentrate, to be diluted with distilled/deionised water; pH 8.0 ± 0.2, yellow coloured solution, bottle with blue cap.
- WASH BUF 20x **Washing Solution (20x conc.) *:** 1 x 50 mL, 20x concentrate, to be diluted with distilled/deionised water, for washing the microtiter wells; pH 7.8 ± 0.2, clear solution, bottle with white cap.
- FEC EXTR BUF 2,5x **Faecal Extraction Buffer (2.5x conc.) **:** 2 x 90 mL, 2.5x concentrate, to be diluted with distilled/deionised water; pH 8.0 ± 0.2, clear solution, bottles with white caps.
- CAL A - F **Calprotectin Standards ***:** 6 vials with 1.0 mL, ready to use; yellow coloured solution, vials with different coloured caps:

| | | |
|---|---|---|
| Standard A: Blue cap | 0 | ng/mL |
| Standard B: Green cap | 7.8 | ng/mL |
| Standard C: Yellow cap | 31.3 | ng/mL |
| Standard D: Red cap | 62.5 | ng/mL |
| Standard E: White cap | 125 | ng/mL |
| Standard F: Black cap | 500 | ng/mL |



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- CTR LOW CTR HIGH **Calprotectin Controls "Low" and "High" ***:** 2 vials with 1.0 mL, ready to use; yellow coloured solution; Ctr Low: vial with brown cap;.Ctr High: vial with purple cap.
- CONJ **Enzyme Conjugate ****:** 13 mL alkaline phosphatase-labelled, immunoaffinity-purified polyclonal rabbit antibodies against Calprotectin, ready to use; red coloured solution, 25 mL Dynex reagent tube with white cap.
- SUB pNPP **Enzyme Substrate Solution (pNPP):** 13 mL**,** ready to use; clear to faint yellow solution, opaque bottle with yellow cap. The bottle contains stabilising pellets.
  *Note*: If using a Dynex instrument, the substrate has to be transferred into a 25mL Dynex reagent tube before running the test.

  * Contains 0.1 % Kathon
  ** Contains <0.1% sodium azide
  *** Contains 0.1 % Kathon and <0.1% sodium azide
  **** Contains 0.02% methylisothiazolone and 0.02% bromonitrodioxane

## 4.2. Materials supplied

- 2 Sealing foils
- 1 Test protocol
- 1 Plate layout

## 4.3. Materials required but not supplied

- Distilled/deionised water
- Extraction devices (see section 7.1.1 and 7.1.2)
- Disposable, breakable inoculation loops (if using weighing method in section 7.1.3)
- Sensitive digital scale (40 – 150 mg) (if using weighing method in section 7.1.3)
- Disposable polystyrene screw cap tubes, 5 mL (if using weighing method in section 7.1.3)
- Vortex mixer
- Disposable tubes for dilution of samples: Eppendorf tubes or similar (if assay is performed manually)
- Pipettes to deliver volumes 10 – 1000 µL (if assay is performed manually)
- Repetitive pipette or multi-channel pipette, 100 µL (if assay is performed manually)
- Microplate well washer or multi-channel pipette, 300 µL (if assay is performed manually)
- Plate shaker (500 – 700 rpm) (if assay is performed manually)
- Timer (if assay is performed manually)
- Microplate reader, filter 405 nm (if assay is performed manually)
- 1M NaOH (stop solution; optional)

# 5. STABILITY AND STORAGE

When stored unopened at 2 – 8°C, kit reagents are stable up to the expiry date stated on the label.

Opened plates, reagents and concentrated buffers are stable for up to three months when stored at 2 – 8°C.

When prepared in clean vessels, working solutions (1x) of Washing Solution, Sample Dilution Buffer and Faecal Extraction Buffer can be stored at 2 – 8°C for up to one month.

Avoid exposure to high temperature and direct sunlight.

# 6. REAGENT PREPARATION

All reagents, samples and controls should be brought to room temperature (18 – 25°C) before starting the test run.


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### 6.1. Coated microtiter plate strips

The ready-to-use plate strips are coated with affinity-purified monoclonal mouse antibodies specific for Calprotectin. Unused strips should be removed from the frame and immediately re-sealed in the aluminium foil pouch along with the desiccant supplied. Store at 2 – 8°C.

### 6.2. Sample Dilution Buffer

Dilute the 5x concentrated Sample Dilution Buffer by adding 1 part (20 mL) to 4 parts (80 mL) distilled/deionised water in a clean vessel to a final volume of 100 mL. Mix well. Store the diluted Sample Dilution Buffer in a closed vessel at 2 – 8°C.

*Note*: If using a Dynex DS2 or DSX ELISA automat, the Sample Dilution Buffer must be transferred to a 25 mL Dynex reagent tube before running the test.

### 6.3. Washing Solution

Dilute the 20x concentrated Washing Solution by adding 1 part (50 mL) to 19 parts (950 mL) distilled/deionised water in a clean vessel to a final volume of 1000 mL. Mix well. Store the diluted Washing Solution in a closed vessel at 2 – 8°C.

### 6.4. Faecal Extraction Buffer

Dilute the 2.5x concentrated Faecal Extraction Buffer by adding 1 part (90 mL) to 1.5 parts (135 mL) distilled/deionised water in a clean vessel to a final volume of 225 mL. Mix well. Store the diluted buffer in a closed vessel at 2 – 8°C.

### 6.5. Standards and controls

The vials labelled with Standard A – F, as well as the controls, contain 1.0 mL each of a ready-to-use solution. The concentration of Calprotectin is printed on the label of each vial. The vials fit directly into Dynex DS2 and DSX ELISA automates.

### 6.6. Enzyme conjugate

The tube contains 13 mL of alkaline phosphatase (ALP)-labelled, immunoaffinity-purified rabbit antibodies against Calprotectin in a buffer with stabilisers, preservatives and an inert red dye. The solution is ready to use. The tube fits directly into Dynex DS2 and DSX ELISA automates.

### 6.7. Enzyme Substrate Solution (pNPP)

The bottle contains 13 mL of *p*-nitrophenylphosphate (pNPP) solution. The solution is ready to use and must be stored in its original, opaque bottle.

*Note*: If using a Dynex DS2 or DSX ELISA automat, the Enzyme Substrate Solution must be transferred to a 25 mL Dynex reagent tube before running the test. Avoid transferring the stabilising pellets.

## 7. SAMPLE COLLECTION AND PREPARATION

The **CALPROLAB™ Calprotectin ELISA (ALP)** has been developed and validated mainly for faecal samples, but can also be used for plasma/serum samples.

### 7.1. Faecal samples

Since Calprotectin is very stable in stools, patients can collect small faecal samples at home. Collect 1 – 5 g (approximately one teaspoonful), place it in a suitable clean container and deliver it to the laboratory as soon as possible but within four days. When put in a container approved for transport, it can be sent by ordinary mail, i.e. no refrigeration is needed. Exposure to temperatures above 30°C should be avoided.

Samples can also be stored frozen, at -20°C or lower, until delivery or mailing. Frozen samples must be thawed and equilibrated to room temperature before extraction and testing. Note that freezing faecal samples can in some cases result in increased Calprotectin levels, most likely due to release from granulocytes.

*Note*: Before commencing extraction, the stool sample should be homogenised well using for example a spatula, before the small amount for extraction is taken out.



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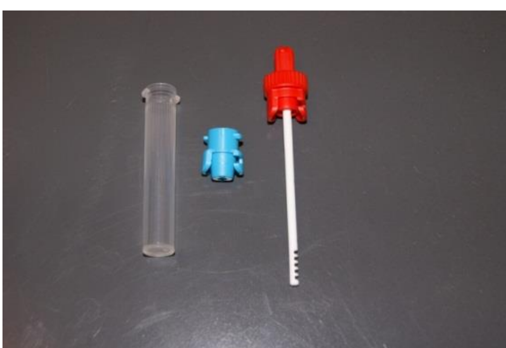
For extraction we recommend the use of Calpro EasyExtract™ or one of the other methods described below (chapter 7.1.2 and 7.1.3). Perform extraction according to package insert for the chosen extraction device/method. Other methods and devices, validated by the customer, can be used.

### 7.1.1. Extraction using the Calpro EasyExtract™

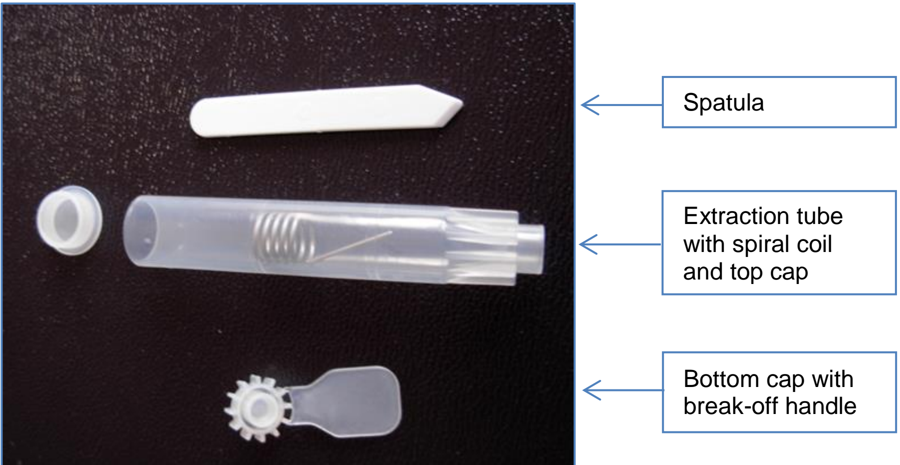
Instructions for use: please read package insert for product No. CAL0510



(Calpro AS, Product No. CAL0510)

### 7.1.2. Extraction using the Faecal Extraction Device

Instructions for use: please read package insert for product No. CAL0500




(Calpro AS, Product No. CAL0500)

### 7.1.3. Extraction using the weighing method (without extraction device)

1. Weigh (tare) an empty screw cap tube with an inoculation loop.
2. Take out approx. 100 mg (between 40 and 120 mg) faeces by means of the inoculation loop and place it into the screw cap tube. Avoid taking out solid, undigested material like fibres and seeds.
3. Weigh tube and loop with faeces which will give the net faeces weight.
4. Break or cut off the top half of the loop handle and leave the bottom part inside the tube.

5. Add extraction buffer to a weight: volume ratio 1:50, for instance 4.9 mL buffer to 100 mg faeces. Close the tube.
6. Mix vigorously for 30 seconds by means of a vortex mixer.
7. Continue the mixing on a shaker (at approx. 1000 rpm) for 30±5 minutes with the loop inside the tube as an agitator.
8. Allow a couple of minutes on the bench for particles to settle and pipette carefully from the top of the tube. No centrifugation is necessary, but a short centrifugation can be performed if a particle-free solution is required.
9. The extract, which represents a 1:50 dilution (weight:volume) of the stool sample, is now ready for dilution and testing.
10. For storage, transfer about 0.5 mL to a new tube. Extracts can be stored at 2 – 8°C for at least five days or frozen below -20°C for up to 2 years [48].

### 7.2. Plasma and serum samples

True levels of Calprotectin in serum or plasma require that the protein is not released from neutrophil granulocytes in blood *in vitro*. For plasma, EDTA is the preferred anticoagulant.

Recommended sample preparation procedure:

1. Collect blood using vacutainers.
2. Centrifuge the blood at 3000 rpm for 10 minutes as soon as possible and no longer than three hours after sampling.
3. Harvest only the upper two thirds of the serum or plasma layer by careful pipetting so that leucocytes from the buffy coat are not aspirated.

For ELISA, dilute the plasma or serum 1:20 (e.g. 50 µl sample + 950 µl Sample Dilution Buffer) and follow the assay procedure described in Section 9.

## 8. SUGGESTED PLATE LAYOUT

| | 1 | 2 | 3 | 4 | etc. | |
|---|---|---|---|---|---|---|
| **A** | Standard F<br>500 ng/mL | Standard F<br>500 ng/mL | Sample 1 | Sample 1 | | |
| **B** | Standard E<br>125 ng/mL | Standard E<br>125 ng/mL | Sample 2 | Sample 2 | | |
| **C** | Standard D<br>62.5 ng/mL | Standard D<br>62.5 ng/mL | Sample 3 | Sample 3 | | |
| **D** | Standard C<br>31.3 ng/mL | Standard C<br>31.3 ng/mL | Sample 4 | Sample 4 | | |
| **E** | Standard B<br>7.8 ng/mL | Standard B<br>7.8 ng/mL | Sample 5 | Sample 5 | | |
| **F** | Standard A<br>0 ng/mL | Standard A<br>0 ng/mL | Sample 6 | Sample 6 | | |
| **G** | Control<br>“Low” | Control<br>“Low” | Sample 7 | Sample 7 | | |
| **H** | Control<br>“High” | Control<br>“High” | Sample 8 | Sample 8 | | |

Suggested ELISA plate layout for standards, controls and samples using manual procedure. Duplicate wells are recommended for increased reliability of results. A full plate takes 40 samples.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## 9. ELISA PROCEDURE

The following procedure is for manual testing. Validated protocols for Dynex DS2 and DSX ELISA automates are available upon request. Please note that the standard and positive control vials, as well as the conjugate tube, fit directly into the DS2 or DSX ELISA automates. Other automated ELISA instruments can also be used, but have to be validated by the customer.

Procedural Notes

- Preparation: Please read the test protocol carefully *before* performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, a plate layout for all standards, samples and controls should be carefully established, using for example the sheet supplied in the kit. Select the required number of microtiter strips. Unused strips should be re-sealed in the aluminium pouch and stored as described in Section 6.1.
- A 1:100 dilution of faeces extracts is recommended. This dilution will give sample results between 25 mg/kg (LoQ) and 2500 mg/kg in faeces. Extracts with higher Calprotectin values can be diluted more (> 1:100) and re-tested if a value is required. Extracts with low Calprotectin values can be diluted less (1:50). The adjusted dilution factor must be taken into account when converting from ng/mL to mg/kg (see section 11 below).
- Perform all assay steps in the order given and without any appreciable delays between the steps.
- A clean, disposable pipette tip must be used for dispensing each standard, control and sample.
- To achieve the most reliable results, standards, controls and patient samples should always be run in duplicate.
- All samples and kit reagents should be equilibrated to room temperature (18 – 25°C) before testing is begun.

ELISA Procedure

1. Dilute faeces extract samples 1:100 (e.g. 10 µl sample + 990 µl Sample Dilution Buffer) and mix well by vortexing.
2. Add 100 µl of each standard, control and diluted sample in duplicate wells; see recommended plate layout in Section 8.
3. Cover the plate with a sealing foil and incubate at room temperature for 40±5 min*) on a horizontal plate shaker (approximately 500 – 700 rpm).
4. At the end of the incubation time, remove the liquid and wash the wells by adding 300 µL Washing Solution to each well. Remove as much liquid as possible and repeat until a total of three washings have been performed. If a plate washer is used, check that all aspirating and filling probes are unblocked to ensure efficient washing of all wells. After the final wash, invert the plate and tap the well openings thoroughly on absorbent tissue to remove any remaining Washing Solution.
5. Mix the content of the Enzyme Conjugate vial gently prior to use (do not shake). Add 100 µl of conjugate to each well, preferably using a repetitive or multichannel pipette.
6. Cover the plate with sealing foil and incubate at room temperature for 40±5 min*) on a horizontal plate shaker (approximately 500 – 700 rpm).
7. Repeat the washing steps as described above, three times with 300 µL Washing Solution per well.
8. Add 100 µl Enzyme Substrate Solution to each well, preferably using a repetitive or multichannel pipette.
9. Incubate the plate at room temperature (without shaking) for 20 – 30 minutes, protected from light.
10. *Optional:* Add 100 µL 1M NaOH stop solution to each well if a fixed incubation period is required.
11. Read the optical density (OD) values at 405 nm using an ELISA reader. If the plate reader has this option, shake the plate briefly (2-3 seconds) before reading.

*) **Optional: Reduced incubation time (manual procedure).** The incubation time can be reduced to 30±5 min without change in the results. A reduction in OD values may be observed, and the maximum OD value for the top standard (500 ng/mL) may fall below the recommended 1.800 but this does not affect the results [46].


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## 10. QUALITY CONTROL

- A new standard curve must be included in each run.
- The positive controls should be included in each run. The value of the controls should be within the limits printed on the vial labels.
- As a guide, the OD value of Standard F (500 ng/mL) should be ≥ 1.6 and the OD value of Standard A (0 ng/mL) should be ≤ 0.25. A representative standard curve is shown in figure 1.

## 11. EVALUATION

Calculation of Calprotectin concentration in patient faecal samples:

1. Calculate the mean OD values of all duplicate wells (standards and samples).
2. Plot the value of each standard concentration (ng/mL) on the x axis against its mean OD value on the y axis to obtain a standard curve. **A 4-parameter curve fit function is recommended** (see figure 1 below). If a logarithmic x axis is required, a value of 0.001 ng/mL must be used for standard A (0 ng/mL).
3. Use the calibration curve to determine the Calprotectin concentration in the diluted samples (ng/mL) based on their OD values.
4. **Multiply the Calprotectin concentration (ng/mL) in the diluted faecal extracts by 5 in order to convert to mg/kg Calprotectin in the original stool sample.**

   This factor corrects for the total dilution of 1:5000 (1:50 during the extraction procedure and the following 1:100 dilution of the extracts) and converts the value from ng/mL to mg/kg.

   *Example: if a diluted extract sample has a value of 100 ng/mL the concentration in the original stool specimen was 100 x 5 = 500 mg/kg.*

   Note: If extracts have been diluted more (or less) than the recommended 1:100, the additional dilution factor must be entered into the calculation.

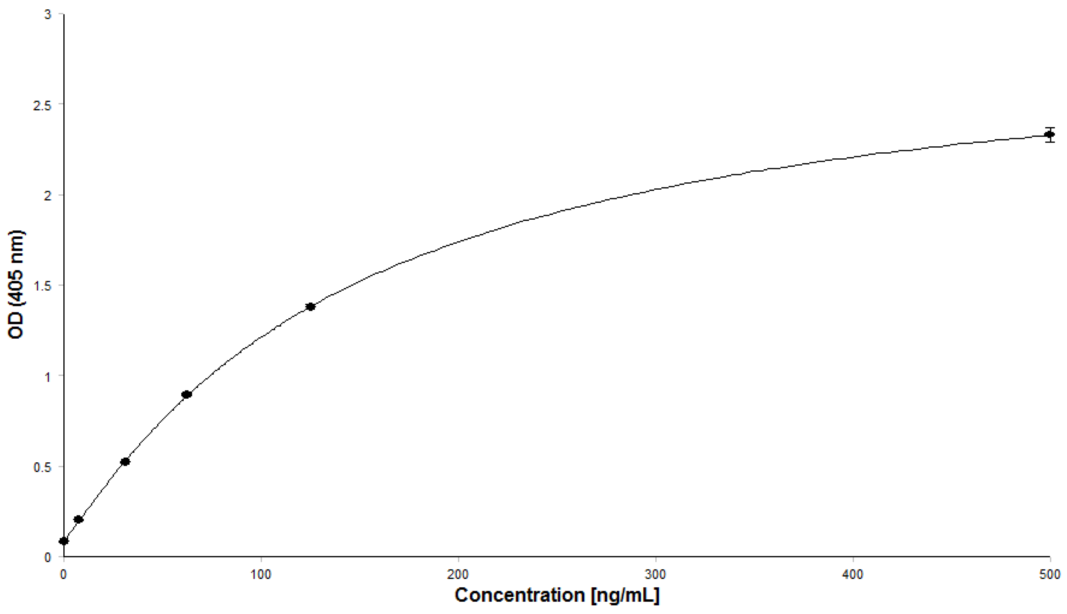


Figure 1: A representative standard curve using 4-parameter curve fit.

## 12. INTERPRETATION OF RESULTS

The following Calprotectin values in stool samples have been reported in the published literature [3, 47)]:

| | |
|---|---|
| Normal value | 5 – 50 mg/kg |
| Positive value | > 50 mg/kg |
| Median value in patients with symptomatic colorectal cancers | 350 mg/kg |
| Active, symptomatic inflammatory bowel disease | 200 – 40,000 mg/kg. |

Note that a diagnosis should not be established based on a single test result. Diagnosis should take into consideration clinical history and symptoms.

The following Calprotectin values were found in blood from 100 healthy blood donors (50 men and 50 women):

| **Sample type:** | **Mean (µg/L):** | **SD:** |
|---|---|---|
| EDTA plasma | 627 | 307 |

## 13. SPECIFICATIONS

Note: All design verification studies were performed by manual testing on faeces extract samples (diluted 1:100) or EDTA plasma samples (diluted 1:20), using the ELISA procedure described in Section 9.

**Inter-assay precision, faeces extracts**

Mean results from three different laboratories, each testing two different kit lots: six samples were tested a total of 10 times over five days:

| **Concentration in faeces (mg/kg)** | **%CV** |
|---|---|
| 32.1 | 13 |
| 171 | 6.0 |
| 368 | 4.8 |
| 583 | 6.1 |
| 1215 | 6.8 |
| 1977 | 7.9 |

**Intra-assay precision, faeces extracts**

Mean results from three different laboratories, each testing two kit lots: six samples were tested with 10 replicates in one run:

| **Concentration in faeces (mg/kg)** | **%CV** |
|---|---|
| 28.7 | 6.7 |
| 173 | 3.6 |
| 385 | 4.1 |
| 592 | 4.0 |
| 1210 | 4.2 |
| 1966 | 5.0 |

**Intra-assay precision, EDTA plasma**

Tested using one kit lot; six samples were diluted 1:20 and tested with 10 replicates in one run:

| **Concentration in plasma (ug/L)** | **%CV** |
|---|---|
| 166 | 3.8 |
| 740 | 4.7 |
| 1160 | 2.9 |
| 1360 | 3.5 |
| 2980 | 3.2 |
| 3660 | 10 |


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**Recovery:**

Faeces: 85 – 105%; tested with faecal extract spiked with purified Calprotectin at five different levels.
Plasma: 81 – 105%; tested with plasma spiked with purified Calprotectin at five different levels.

**Limit of Quantification:**

5 ng/mL; tested with faeces extract, plasma and purified Calprotectin. Samples were analysed five times over five days. The mean CV for the different samples and determinations at this level was 12%.

**Limit of Detection:**

< 5 ng/mL; calculated as mean (Sample Dilution Buffer; n= 32) + 5x SD.

**Agreement with Calpro Calprotectin ELISA CAL0100**

Satisfactory correlation and agreement has been found between samples analysed in CAL0100 and in CALP0170:

Faeces extracts: $R^2 = 0.792$ (48 – 1250 mg/kg, n=118 samples)
$R^2 = 0.864$ (48 – 500 mg/kg, n=85 samples)

Plasma: $R^2 = 0.977$ (260 – 6400 µg/L, n=16 samples)

**Interference**

No observed interference on the ELISA from commonly used pharmaceuticals: Prednisolon, Imurel, Salazopyrin and Ciprofloaxcin.

**Extraction precision**

Two faeces samples were extracted 10 times each, using the procedure described in section 7.1.2, and the extracts analysed in the ELISA. Mean results from three different laboratories and one kit lot:

| Sample | Concentration (mg/kg) | %CV |
|---|---|---|
| Low | 130 | 8.7 |
| High | 1357 | 8.8 |

**Linearity, faecal extracts**

Faecal extracts (n=10) were diluted 1:100 – 1:1000 and analysed in the ELISA. Mean results:

| Dilution | % of 1:100 dilution |
|---|---|
| 1:100 | 100 |
| 1:400 | 103 |
| 1:700 | 105 |
| 1:1000 | 107 |

Note that sample variation in linearity has been observed.

**Linearity, EDTA plasma**

Plasma samples (n=5) were diluted 1:20 – 1:80 and analysed in the ELISA. Mean results:

| Dilution | % of 1:20 dilution |
|---|---|
| 1:20 | 100 |
| 1:40 | 107 |
| 1:80 | 116 |

Note that sample variation in linearity has been observed.

## 14. LIMITATIONS OF THE PROCEDURE

- Diagnosis should not be established based on a single test result. Diagnosis should take into consideration clinical history and symptoms.


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## 15. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the *in vitro* diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analysers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for *in vitro* diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and hepatitis B antigen (Bag) and have been found to be non-reactive. Nevertheless, all materials should be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- Do not use reagents from other manufacturers with reagents of this test kit.
- Do not use reagents after expiry date stated on the label or after 1 months of preparation of concentrated reagents to working solutions.
- Use only clean pipette tips, dispensers, and lab ware.
- To prevent cross contamination, do not interchange screw caps of reagent vials.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage, check conjugate, standards and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results, pipette standards, control and faecal extract samples, and dispense conjugate and substrate, accurately to the bottom of microplate wells, without splashing..
- Some reagents contain sodium azide at less than 0.1% (w/v) and/or 0.1% Kathon.
- Store the substrate solution in the original, opaque bottle; the solution should be clear to pale yellow. Mix gently before use.
- The **CALPROLAB™ Calprotectin ELISA (ALP)** is designed for use by qualified personnel who are trained in good laboratory practice.

### Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

## 16. REFERENCES


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## 17. ORDER INFORMATION

Product code: CALP0170 CALPROLAB™ Calprotectin ELISA (ALP) (96 Determinations)

| Symbols Key / Symbolschlüssel / Tabela de símbolos | |
|---|---|
| | Manufactured by / Hergestellt von / Fabricado por / Fabricado por |
| IVD | In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Producto para diagnóstico in vitro |
| LOT | Lot Number / Chargenbezeichnung / Número de lote |
| | Expiration Date / Verfallsdatum / Data de Validade |
| | Storage Temperature / Lagertemperatur / Temperatura de almacenamiento |
| CE | CE Mark / CE-Zeichen / Marca CE |
| REF | Catalogue Number / Katalog Nummer / Número de Catálogo |
| i | Catalogue Number / Katalog Nummer / Número de Catálogo |
| MTP | Microplate / Mikrotiterplatte / Microplaca |
| CONJ | Conjugate / Konjugat / Conjugado |
| CAL | Calibrator A-F / Kalibrator A-F / Calibrador A-F |
| CTR LOW | Control Low / Kontrolle Niedrig / Control Bajo |
| CTR HIGH | Control High / Kontrolle Hoch / Control Alto |
| DIL 5x | Sample diluent buffer 5x concentrated / Probenverdünnungspuffer 5x konzentriert / Solución tampón para muestras concentrado x5 |
| SUB pNPP | pNPP Substrate solution / pNPP-Substratlösung / Solución substrato pNPP |
| FEC EXTR BUF 2,5x | Faecal Extraction Buffer 2,5x concentrated / Stuhlextraktionspuffer 2,5x konzentriert / Buffer fecal de extracción (2,5 x conc.) |
| Σ n | Contains sufficient for “n” tests / Ausreichend für “n” Tests / Contenido suficiente para ”n” tests |


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# QUICK GUIDE

## CalproLab™ ELISA (ALP) for analysis of Calprotectin in faeces

*Please refer to sections 7 – 9 in the package insert for a full description of the practical steps and for analysis of plasma/serum.*

## Extraction

- ❑ Perform extraction according to one of the methods described in section 7.1.1 – 3

## ELISA (manual procedure)

- ❑ Dilute faecal extracts 1:100 in Sample Dilution Buffer
- ❑ Add 100 µL standards, controls and samples to the ELISA plate
- ❑ Incubate on a plate shaker at room temperature for 40±5 min*)
- ❑ Wash the wells three times with 300 µL Washing Solution
- ❑ Add 100 µl of ALP enzyme conjugate to each well
- ❑ Incubate on a plate shaker at room temperature for 40±5 min*)
- ❑ Wash the wells three times with 300 µL Washing Solution
- ❑ Add 100 µL pNPP Enzyme Substrate Solution to each well
- ❑ Incubate under cover for 20 – 30 min
- ❑ *Optional:* add 100 µL 1M NaOH to each well
- ❑ Read the OD values at 405 nm using an ELISA reader
- ❑ Using a 4-parameter curve fit, calculate the results (ng/mL)
- ❑ mg/kg in faeces = ng/mL × 5

*) can be reduced to 30±5 min if the assay is run manually.

For questions, please contact mail@calpro.no


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