



Calpro Easy Extract™

One-step sampling and extraction device



1. INTENDED USE

Calpro EasyExtract™ is intended for extraction of stool samples for measurement of Calprotectin in humans.

This device has been validated in combination with CalproLab™ Calprotectin ELISA (CALP0170).

The device is for *in vitro* diagnostic use.

The device can only be used once.

2. MATERIALS

One box of extraction devices (product No. CAL0510) contains 50 EasyExtract™ devices pre-filled with Calpro faecal extraction buffer. The devices are ready to use.

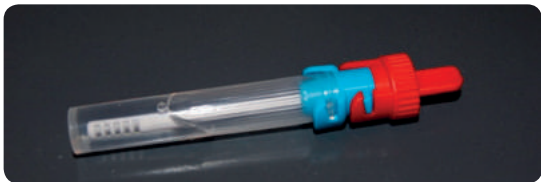


Figure 1: Calpro EasyExtract™

The extraction device consists of:

- A** a tube filled with buffer
- B** a **blue adaptor** (cap) screwed to the tube
- C** a **red cap** with a sample rod (white stick with grooves in the end)

3. STABILITY AND STORAGE

3.1. Unopened EasyExtract™ device

The buffer in the extraction device is stable for following duration of time at given temperatures:

2-8°C	20-25°C	37°C	45 °C
18 months	12 weeks	12 weeks	2 weeks

The stability is subject to storage conditions and expiry date of the lot. The tubes should be stored standing in cold storage for optimal stability, and it is important to avoid direct sunlight.

3.2. EasyExtract™ device with faecal extract

The stability of the extraction device with fecal extract is given at various temperatures:

-20°C	2-8°C	20-25°C	37°C
12 months	9 days	7 days	3 days

The stability of calprotectin stated in the table is the longest achievable stability if the samples were stored at the stated temperature right after the extraction.. A sample that for instance has been kept at room temperature for 4 days, will not be stable in fridge for 9 days after that. When samples are received where home extraction is used, it is advisable to place the sample in 2-8°C if they are to be analysed within 2 days and -20°C if they are to be analysed later than that. This is to make sure that the duration of sample storage does not exceed recommended duration.

Frozen samples can be frozen/thawed 3 times without affecting their Calprotectin levels significantly.

If extracts are to be stored in the freezer for longer than 6 months, it is recommended to transfer the extracts into cryotubes.

4. EXTRACTION PROCEDURE

4.1 General procedure

The procedure for extraction of Calprotectin using the Calpro EasyExtract™ device is described and illustrated below:

- 1 Take out the required amount of EasyExtract™ devices from the box. The tubes can be used directly without having them equilibrate to room temperature before use.
- 2 Hold the blue adaptor in place and release the stick by rotating **the red cap** counter-clockwise (*figure 2*).
- 3 Take out the white stick, attached to **the red cap**.
- 4 Place the stick into the stool sample and stir around to get a selective sample. Make sure that the grooves of the stick are completely filled with stool. Grains and seeds must be avoided. Also avoid trapping air bubbles (*figure 3*). It is advisable to have stool in excess around the grooves when re-inserting the rod into the tube.
- 5 Return the stick through the hole in **the blue adaptor** on the tube. By inserting the stick into the tube, excess stool is wiped off in the funnel insert. Push together until it stops (*figure 4 and 5*).
- 6 Turn **the red cap** clockwise until it clicks into the locked position. Make sure that both **the blue adaptor** and **the red cap** are in the locked position (*figure 6*).
- 7 Vortex the assembled device vigorously for about three minutes to disrupt large particles. The stool material should be suspended completely in extraction buffer. If necessary, furt-

her vortexing may be performed so that grooves are free of faeces and larger particles are broken down (*figure 7 and 8*).

- 8 If the samples are to be analysed the following day or later, they can be stored after performing step 7. However, it is then important to repeat step 7 prior to analysis to ensure the homogeneity of the preparation. Skipping the vortexing before the analysis can lead to measuring false low levels of calprotectin. Extracts can be stored in the extraction tube at 2 – 8°C for up to five days or frozen below -20°C for up to six months¹⁾.
- 9 Samples can safely be stored for analysis at later point. For information on extract stability, please refer to 3.2 in this booklet
- 10 The extract is now ready for dilution according to the package insert of the assay to be used. The extract represents a 1:50 dilution (weight:volume) of the stool sample.
- 11 For analysis, open the tube by twisting **the blue adaptor** counter-clockwise and removing both the adaptor, **the red cap** and the stick (*figure 9*).



Figure 2

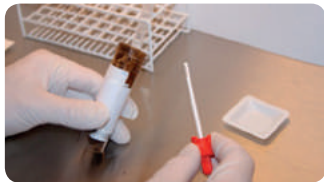


Figure 3

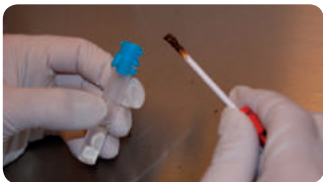


Figure 4



Figure 5



Figure 6

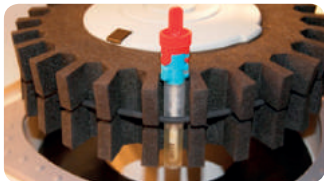


Figure 7



Figure 8



Figure 9

4.2 Practical advice when using Easy Extract

- It is not necessary to have morning stool sample for extraction, but the duration from last bowel movement until the one the sample is taken from will have a say in levels of Calprotectin.
- Calprotectin in stool can be unevenly distributed, thus by stirring around the sample a more correct result will be obtained
- Samples that are very fluid will still contain Calprotectin if present, however these samples can be very difficult to collect using the sample rod. In these cases, it is recommended to apply 30 μ l of sample using a pipette. In case a pipette is used, it might be easier to get 30 μ l of sample if the tip of the pipette tip is trimmed using scissors.
- The grooves can be difficult to fill if the feces is too hard. In this case, it will be helpful to force the groove end of the rod into the stool sample and using it to “scrape” the sample into the grooves. Repeat this at various location in the sample to get a representative extraction.

5. PERFORMANCE

5.1 Precision I: repeated extractions from homogenised stool samples

Five parallel extractions were performed from four different homogenised stool samples with different Calprotectin levels. The extracts were analysed using the CalproLab™ Calprotectin ELISA (CALP0170). The results are shown in table 1.

Sample ID	Mean	CV%	Max	Min
A	41	8.8	45	36
B	670	5.6	715	630
C	1290	8.6	1425	1120
D	1675	6.1	1840	1590

Table 1: Mean Calprotectin concentration (mg/kg) coefficient of variation (%CV), max and min of four stool samples.

5.2 Precision II: spot variation using EasyExtract™

Five different routine stool samples were extracted five times each at two external laboratories using the EasyExtract™ device. The extracts were analysed using the CalproLab™ Calprotectin ELISA (CALP0170). The results for all 10 samples are shown in table 2.

Sample No.	1	2	3	4	5	6	7	8	9	10
Extract 1	<25	163	611	992	2135	134	104	382	830	1313
Extract 2	<25	94	560	954	1958	135	128	360	989	1193
Extract 3	<25	122	453	1118	1722	167	99	380	853	914
Extract 4	<25	101	465	1095	1988	152	120	399	760	1100
Extract 5	<25	105	439	1089	2139	102	125	363	667	1239
Mean	-	117	506	1050	1988	138	115	377	820	1152
%CV	-	24	15	6,9	8,6	18	11	4,2	15	13

Table 2: Calprotectin levels (mg/kg) in 10 different stool sample that was extracted with the EasyExtract™. Each stool sample was extracted five times.

5.3 Comparison of Calpro EasyExtract™ and the original weighing method.

Selected patient stool samples (n=52) were extracted using the EasyExtract™ devices according to the procedure described in Section 4. In parallel, the same stool samples were extracted using the original weighing method²⁾.

The samples varied both in concentration and texture.

The extracts were measured with the CalproLab™ Calprotectin ELISA (CALP0170). The results obtained with the two different extraction methods were equivalent (*figure 10*).

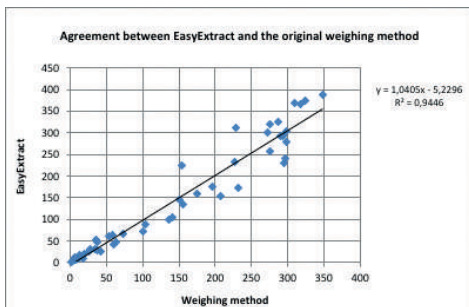


Figure 10: Calprotectin levels (ng/mL) in stool extracts prepared with Calpro EasyExtract™ (CAL0510) and the original weighing method. All samples were measured with CalproLab™ Calprotectin ELISA (CALP0170).

6. LIMITATIONS OF THE PROCEDURE

When carefully following the procedure, approximately 30 mg faeces will be extracted. For very fluid samples, the sample vial can be filled using a pipette (take out 30 µL fluid sample). Please note that Calprotectin is present in the fluid as well as the solid part of stools.

Calprotectin in stool may not be evenly distributed throughout the sample. Even after homogenisation of the sample, spot variations can occur³⁾.

Do not use Calpro EasyExtract™ devices after the expiry date or if there are signs of microbial contamination.

7. SAFETY AND PRECAUTIONS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the *in vitro* diagnostic medical devices is intended 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. Any change in design or test procedure as well as any use outside the intended and 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. Only for *in vitro* diagnostic use.
- Handling of stool samples should be performed inside an appropriate cabinet using lab coat and gloves to protect against possible infections and microbial contamination. Area used for sample handling should be cleaned with an anti-microbial liquid after use.

8. DISPOSAL CONSIDERATIONS

Residues of patient samples and extracts are generally considered as biological 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 biological hazardous waste.

9. REFERENCES

1. Tøn H et al.: Improved assay for fecal calprotectin. *Clinica Chimica Acta* 2000; 292: 41-54.
2. Package insert for CalproLab Calprotectin ELISA, prod. No. CALP0170 and CALP0270 (Calpro AS)
3. Røseth et al.: Assessment of the neutrophil dominating protein calprotectin in feces. *Scand J Gastroenterol* 1992;27:793-798.
4. Acevedo et al.: Fecal Calprotectin: A Comparison of Two Commercial Enzymeimmunoassays and Study of Fecal Extract Stability at Room Temperature. *Journal of Clinical Medical Research* 2018;10:396-404

10. ORDERING INFORMATION

Product No.: CAL0510 (50 devices)

Order from: CALPRO AS, Arnstein Arnebergsvei 30, 1366 Lysaker, Norway
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Version 04-september 2018



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