

CALPRO
EASY EXTRACT™

Quick and easy stool sample collection and
extraction for Calprotectin measurement

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1. INTENDED USE

Calpro Easy Extract™ is intended for sampling and extraction of Calprotectin from human stool samples. Sampling can be performed by patient or by clinical/ laboratory staff at the hospital/lab.

This device has been validated in combination with CalproLab™ Calprotectin ELISA and CalproGold Turbidimetric assay.

The device is for *in vitro* diagnostic use. The device can only be used once.

2. MATERIALS



Calpro Easy Extract™

The extraction device consists of

- A. A tube filled with extraction 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 Easy Extract™ device

The buffer in the extraction device is stable for following duration of time at given temperatures and subject to the expiry date of the lot:

2-8°C	20-25°C	37°C	45 °C
Until expiry date	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 Easy Extract™ device with faecal extract

Stability of fecal extracts to be analyzed with CalproLab or CalproGold is shown below at various storage conditions:

-20°C	2-8°C	20-25°C
12 months	7 days	5 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 at 2-8°C for 7 days after that. Extracted samples sent to the lab by mail should be placed at 2-8°C if they are to be analyzed within 24 hrs and -20°C if they are to be analyzed later than that. This is to make sure that the duration of sample storage does not exceed recommended duration.

Frozen samples can be freeze - 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 Easy Extract™ device is described and illustrated below:

1. Take out the required amount of Easy Extract™ devices from the box. The tubes can be used directly without the need to equilibrate to room temperature.
2. Hold the blue adaptor in place and release the stick by rotating **the red cap** counter-clockwise (*Img 1*).
3. Take out the white stick, attached to **the red cap**.
4. Place the stick into the stool sample and mix around to get a representative sample. Make sure that the grooves of the stick are filled. Grains and seeds must be avoided. Also avoid trapping air bubbles. 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 (*Img 2 and 3*).
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 (*Img 4*).
7. Vortex the assembled device vigorously for about three minutes to disrupt large particles. The stool should be suspended completely in the extraction buffer. If

necessary, further vortexing may be performed so that grooves are free of faeces and larger particles are broken down (*Img 5 and 6*).

If the samples are to be analyzed the following day or later, they can be stored according to table in 3.2 after vortexing. It is then important to repeat vortexing prior to analysis to ensure the homogeneity of the preparation. Skipping the vortexing before the run can lead to measuring lower levels of calprotectin.

Samples can safely be stored for analysis at later point. For information on extract stability, please refer to 3.2 in this booklet.

The extract represents a 1:50 dilution (weight/volume) of the stool sample. It can now be further diluted according to the package insert of the assay to be used.

For analysis, open the tube by twisting **the blue adaptor** counterclockwise and removing both the adaptor, **the red cap** and the stick.



Img 1



Img 2



Img 3



Img 4



Img 5



Img 6

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 µl of sample using a pipette.
- If patient is extracting at home, then samples not collectable with the sampling rod must be sent to the lab for sampling.
- The grooves can be difficult to fill if the feces are 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.

4.3 Sampling by patient

EasyExtract can be used for sampling by patient subject to proper training by the clinic/hospital. If the patient is to send the collected sample to the clinic/hospital, the latter will also need to supply suitable containers for shipment.

If patient send sample by mail, it is important to keep in mind the stability limitations specified in chapter 3.2.

5. PERFORMANCE

5.1 Precision

6 stool samples from 47 up to 758 mg/kg Calprotectin were extracted 5 times by three different operators. This was repeated for five days giving in total 75 separate extracts per sample. The extracts were analyzed using both CalproLab™ Calprotectin ELISA and CalproGold turbidimetric assay. The results are shown per assay in the two tables below:

Extraction precision using Calprolab ELISA

Sample	Mean (mg/kg)	Repeatability (%CV)	Reproducibility (%CV)	Between operators (%CV)
1	46,7	8,8	12,6	3,6
2	125	9,9	13,4	6,7
3	134	8,7	13,7	3,1
4	350	10,6	16,9	2,2

5	585	6,7	10,6	2,1
6	758	10,0	12,9	0,0

Extraction precision using CalproGold turbidimetric assay

Sample	Mean (mg/kg)	Repeatability (%CV)	Reproducibility (%CV)	Between operators (%CV)
1	51,9	8,9	16,5	4,5
2	127	9,5	11,6	2,4
3	159	6,4	12,5	3,2
4	361	9,0	12,2	1,5
5	473	4,8	11,9	0,0
6	694	3,9	6,2	3,0

5.1 Sample uptake

An aliquot from 30 samples with different texture (2 to 6 according to Bristol Stool Chart) were taken using the Easy Extract sampling rod and the blue adaptor. Mean weight of feces collected in the grooves of the sampling rod was 29.2 mg with a % CV of 2.7.

5.2 Comparison to 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*).

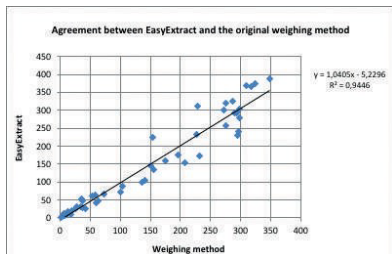


Figure 10: Calprotectin levels (ng/mL) in stool extracts prepared with Calpro Easy Extract™ (CAL0510) and the original weighing method. All samples were measured with CalproLab™ Calprotectin ELISA (CALP0170).

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 homogenization of the sample, spot variations can occur³⁾.

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

Sampling by patient must be organized by the relevant health institution themselves.

6. SAFETY AND PRECAUTIONS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the *in vitro* diagnostic medical devices are 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 must 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.

7. 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.












8. REFERENCES

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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 Enzymoimmunoassays and Study of Fecal Extract Stability at Room Temperature. *Journal of Clinical Medical Research* 2018;10:396-404

10 ORDERING INFORMATION

CAL0510	50 devices
CAL0510L	50 devices labelled with lot and expiry date on each tube

Product code: CAL0510, CAL0510L

Symbols Key / Symbolschlüssel /Tabela de símbolos	
	Produsert av/Manufactured by / Hergestellt von / Fabricado por / Fabricado por
	In Vitro Diagnostisk medisinks utsyr/In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Producto para diagnóstico in vitro
	Lot nummer/Lot Number / Chargenbezeichnung / Número de lote
	Holdbarhetsdato/Expiration Date / Verfallsdatum / Data de Validade
	Lagringstemperatur/Storage Temperature / Lagertemperatur / Temperatura de almacenamiento
	CE merke/CE Mark / CE-Zeichen / Marca CE
	Katalognummer/Catalogue Number / Katalog Nummer / Número de Catálogo
	Catalogue Number / Katalog Nummer / Número de Catálogo
	Inneholder nok for n antgall tester/Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenido suficiente para "n" tests
	Do not reuse/nicht wiederverwenden/ ne pas réutiliser/ non riutilizzare/ no reutilizar
	This side up/Diese Seite oben/ce côté vers le haut/questo lato in su/Este lado hacia arriba

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