

Faecal Extraction Device:

1: Intended use

The Faecal Extraction Device is intended for extraction of stool samples for measurement of biological markers.

This device has been validated for in combination with **Calpro Calprotectin ELISA** measuring Calprotectin in human stools.

The device is for *in vitro* diagnostic use.

The device can only be used once.

2: Materials

One box of extraction devices (product no. CAL500) contains:

- 50 extraction tubes with spiral coil and top cap
- 50 bottom caps with sample beaker and break-off handle
- 50 spatulas

Top cap

Spatula

Tube with spiral coil



Bottom cap with a beaker and handle

3: Extraction Procedure

The Faecal Extraction Device is assembled during the extraction procedure as described and illustrated by the pictures below:

1. Use the small spatula provided to fill the beaker. Alternatively, an inoculation loop can be used. The beaker will take about 100 mg sample. Grains and seeds should be avoided. Also avoid trapping air bubbles.
2. Wipe off excess sample and push the cap *tightly* into the cone of the extraction tube lower end.
3. Fill the extraction tube with 4.9 ml extraction buffer and attach the top cap.
4. Vortex the assembled device vigorously for about three minutes to disrupt large particles. If necessary, further vortexing may be performed so that only solid particles remain.
5. Allow particles to settle by leaving the tube on the bench for a couple of minutes. No centrifugation is necessary, but a short centrifugation can be performed if a particle-free solution is required.
6. Transfer about 0.5 mL to a new tube for assay or storage. Extracts can be stored at 2 – 8°C for at least five days or frozen below -20°C for up to six months ¹⁾.
7. 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.



1: Fill the beaker with sample and wipe off excess sample



2: Fasten the cap tightly to the bottom part of the tube



3: Fill the tube with 4.9 mL Extraction Solution



4: Vortex vigorously (1000 rpm) for three minutes.

The extract is now ready after settlement of particles in the supernatant.

4: Performance

Variation in mg stool sample when using the Faecal Extraction Device

30 spot samples from one pooled stool portion were extracted in parallel using the Faecal Extraction Device and analysed for Calprotectin by Calpro Calprotectin ELISA (Calpro AS). Table 1 shows both variation in mg faeces that was filled in the beaker and the variation in measured Calprotectin in the resulting extract.

The amount of faeces filled into the beaker was found to be close to 100 mg with very little variation between the different devices. Also, there was very little variation in the Calprotectin levels in the resulting extracts.

Table 1: Variation in mg stool sample and Calprotectin levels using the Faecal Extraction device with 30 independent extractions.

	Amount of faeces in beaker (mg)	Calprotectin concentration in extract (mg/kg) *)
Mean	96	919
SD	6	48
%CV	5,8	5,2
Max	106	1020
Min	85	830

*) test results in Calpro Calprotectin ELISA (CAL0100)

Comparison between the original weighing method² and the Faecal Extraction Device

Selected patient stool samples (n = 18) were extracted using the original extraction procedure² with weighing of a faecal aliquot, extraction using vortexing and shaking and high-speed centrifugation.

In parallel, the same stool samples were extracted using the Faecal Extraction Device. The extracts were not centrifuged.



All extracts were the measured with the Calpro Calprotectin ELISA, HRP (CAL0300) *). The results are shown in figure 1. The results obtained with the Faecal Extraction Device were equivalent to the original weighing procedure with centrifugation.

*) The extracts were also tested with the Calpro Calprotectin ELISA, ALP (CAL0100) and CalproLab Calprotectin ELISA (CALP170) giving similar results (data not shown).



Figure 1:

Calprotectin levels in stool extracts prepared by with weighing, extraction and centrifugation (10000 x g, 20 minutes), and extracts prepared by using the Calpro Extraction Device. All samples were measured with Calpro Calprotectin ELISA HRP (CAL0300)

5: Limitations of the procedure

When carefully following the procedure, approximately 100 mg faeces will be extracted. For fluid samples, the sample beaker can be filled by using a pipette. Please note that Calprotectin is present in the fluid as well as the solid part of stools. If an exact amount of sample is needed, the bottom cap must be weighed before and after filling of the beaker.

Calprotectin in stool may not be evenly distributed throughout the sample. Even after homogenization of the sample, spot variations can occur.³



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 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.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.

The Faecal Stool Extraction Device is designed for use by qualified personnel who are trained in good laboratory practice

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: Rereferences

1. Tøn H et al.: Improved assay for fecal calprotectin. Clinica Chimica Acta 2000; 292: 41-54.
2. Package insert for Calpro Calprotectin ELISA, prod. No. CAL0100 and CAL0300 (Calpro AS)
3. Røseth et al.: Assessment of the neutrophil dominating protein calprotectin in feces. Scand J Gastroenterol 1992;27:793-798.

9: Ordering information

Product No.: 500 (50 devices)

Order from: CALPRO AS, Arnstein Arnebergsvei 30, 1366 Lysaker, Norway

Tel: + 47 40 00 42 79

mail@calpro.no

www.calpro.no