Calpro EasyExtract™

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

When stored unopened at 2 – 8°C, EasyExtract™ is stable up to the expiry date stated on the label.

Avoid exposure to high temperature and direct sunlight.

3.2 EasyExtract™ device with faecal extract

Calprotectin is found to be stable in extraction solution up to five days at room temperature (18 – 25°C).

After use, EasyExtract™ devices with faecal extract can be transported for up to five days without cooling as long as the temperature stays below 25°C.

Recommended storage of EasyExtract™ devices with faecal extract is up to five days at 2 – 8°C or frozen below -20°C for up to six months[1)].

4: Extraction procedure

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

1. Take out the required amount of EasyExtract™ devices from the box and equilibrate to room temperature before use.
2. Hold the blue adaptor in place and release the stick by rotating the red cap counter-clockwise (see figure 2).
3. Take out the white stick, attached to the red cap.
4. Dip the stick into the stool sample in three different places, making sure that the grooves of the stick are filled with stool. Grains and seeds should be avoided. Also avoid trapping air bubbles (see figure 3).
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 (see 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 (see 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, further vortexing may be performed so that only solid particles remain and the grooves are free of faeces (see figure 7 and 8).
8. Allow particles to settle by leaving the tube on the bench for a couple of minutes. A short centrifugation can be performed if a particle-free solution is required.
9. 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 months[1)].
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 (see figure 9).

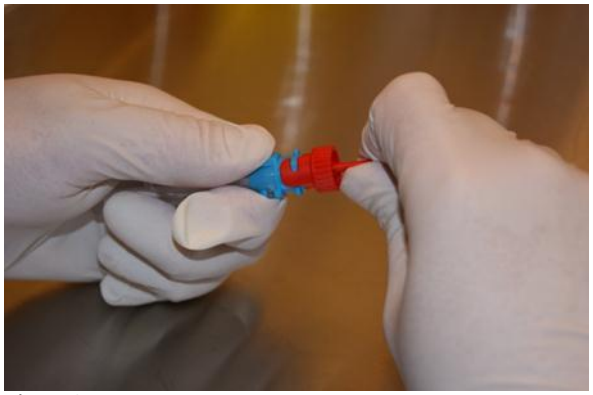
Figure 2

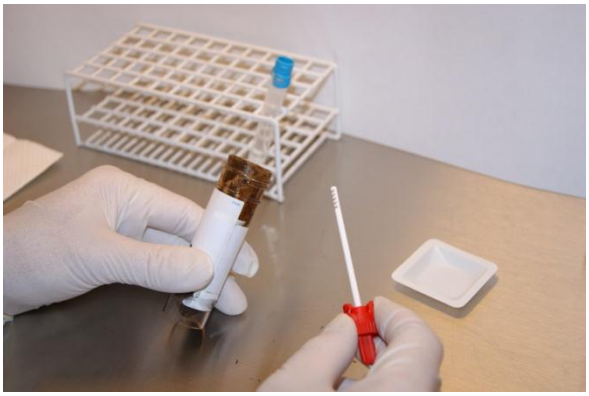
Figure 3


Figure 4

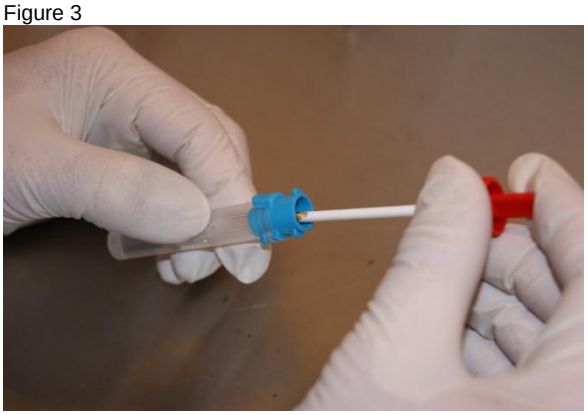
Figure 5


Figure 6

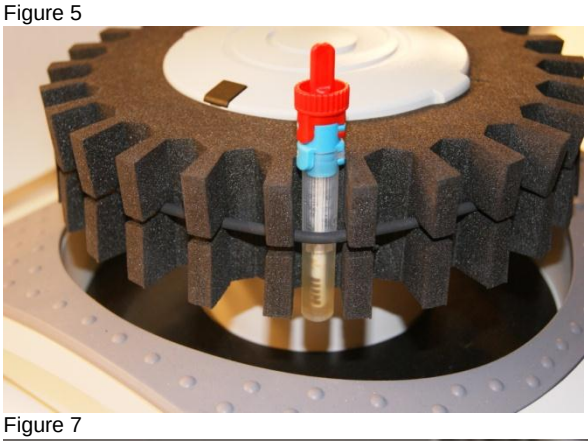
Figure 7


Figure 8


Figure 9

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.

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

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

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.

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

Sample No.	Extract 1	Extract 2	Extract 3	Extract 4	Extract 5	Mean	%CV
1	*<25*	*<25*	*<25*	*<25*	*<25*	-	-
2	163	94	122	101	105	117	24
3	611	560	453	465	439	506	15
4	992	954	1118	1095	1089	1050	6.9
5	2135	1958	1722	1988	2139	1988	8.6
6	134	135	167	152	102	138	18
7	104	128	99	120	125	115	11
8	382	360	380	399	363	377	4.2
9	830	989	853	760	667	820	15
10	1313	1193	914	1100	1239	1152	13

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 method[2)].

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 (see figure 10).

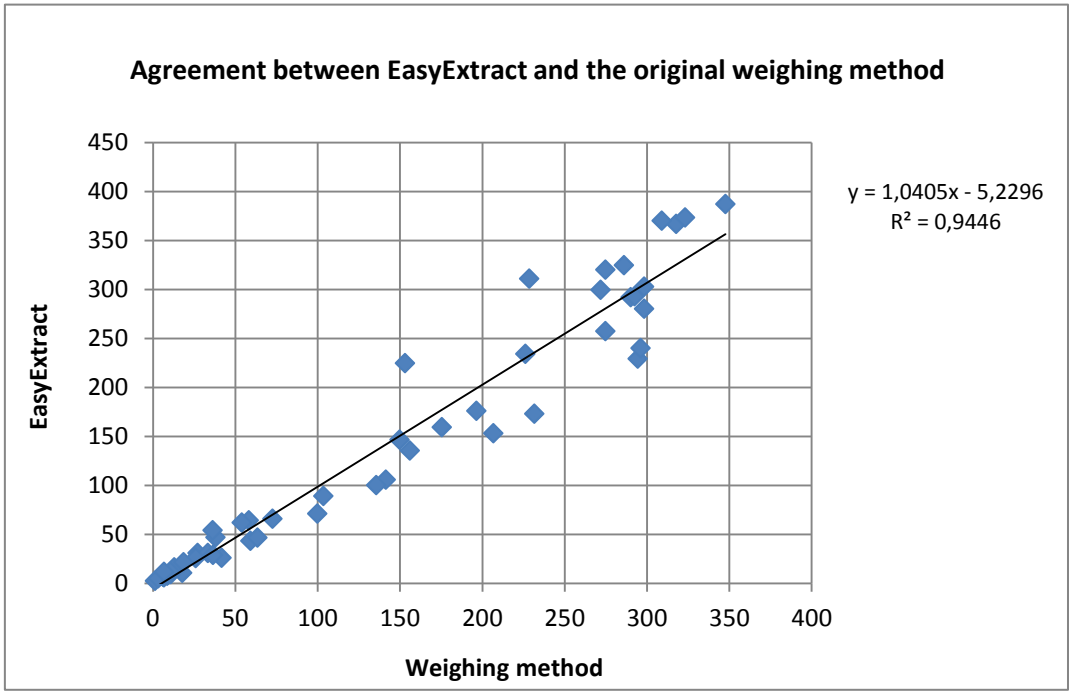

Figure 10: Calprotectin levels (ng/mL) in stool extracts prepared with Calpro EasyExtract™ (CAL0510) and the original weighing method. All samples were measured with CalproLab™ Calprotectin ELISA (CALP0170).

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 occur[3)].

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.

10: Ordering information

Product No.: CAL0510 (50 devices)

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

Tel: + 47 40 00 42 79

mail@calpro.no

www.calpro.no