

INFORMATION LETTER

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Simplified Extraction Procedure for Calprotectin ELISA kits

Background: In the package insert of our ELISA (product numbers CAL0100 and CAL0300), the current faecal extraction procedure consists of three major steps:

- 1) Weighing of a sample aliquot and addition of Faecal Extraction Buffer,
- 2) Extraction by vortex mixing and subsequent shaking,
- 3) High speed centrifugation at low temperature.

Methods: In order to investigate the need for high speed centrifugation, we have performed a series of internal studies. For this purpose we used both pooled samples and selected patient stool samples with various Calprotectin levels within the measuring range of our ELISA kits.

Three different centrifugation procedures were chosen:

- A. Centrifugation for 20 minutes at 10 000 x g, 2 – 8°C (current procedure according to package insert)
- B. Centrifugation with a bench top centrifuge for 10 minutes at 5000 rpm, room temperature.
- C. No centrifugation. Particles in the extracts were allowed to settle for 10-15 minutes.

Extracts were analysed with the Calpro Calprotectin ELISA (CAL0100 and CAL0300).

Results: No significant difference was observed between samples extracted with method A, B and C.

Examples of the results with 9 different stool samples tested with Calpro Calprotectin ELISA HRP (CAL0300) are given in table 1. Figure 1 shows correlation between Method A (10 000 x g) and Method C (no centrifugation), testing 12 different extracts with Calpro Calprotectin ELISA ALP (prod. no CAL0100).

Conclusion/recommendations: No centrifugation of the extract is necessary. If a particle-free extract is required, a short centrifugation with a bench top centrifuge can be used.

The package inserts for product number CAL0100 and CAL0300 will shortly be updated according to these recommendations.

For questions or comments about this, please feel free to contact us at mail@calpro.no or phone +47 4000 4279.

Table 1:

Extraction of nine different stool samples with three different centrifugation procedures. Calprotectin levels in the extract samples were measured with Calpro Calprotectin ELISA HRP (CAL0300). Values in ng/mL.

Faecal sample ID	Method A (10 000xg, 2 – 8°C)	Method B (5000 rpm, room temp.)	Method C (no centrifugation)
0310-18	10	10	< 7.8
0310-21	< 7.8	< 7.8	< 7.8
0310-89	60	59	65
0310-64	97	110	109
0310-54	76	76	79
0310-128	84	76	91
0310-5	< 7.8	< 7.8	< 7.8
230310-5	302	345	284
0310-141	88	88	98

Figure 1:

Extraction of 12 different stool samples with centrifugation at 10.000 x g or with no centrifugation. Calprotectin levels in the extract samples were measured using Calpro Calprotectin ELISA ALP (CAL0100).