BÜHLMANN fCAL® ELISA

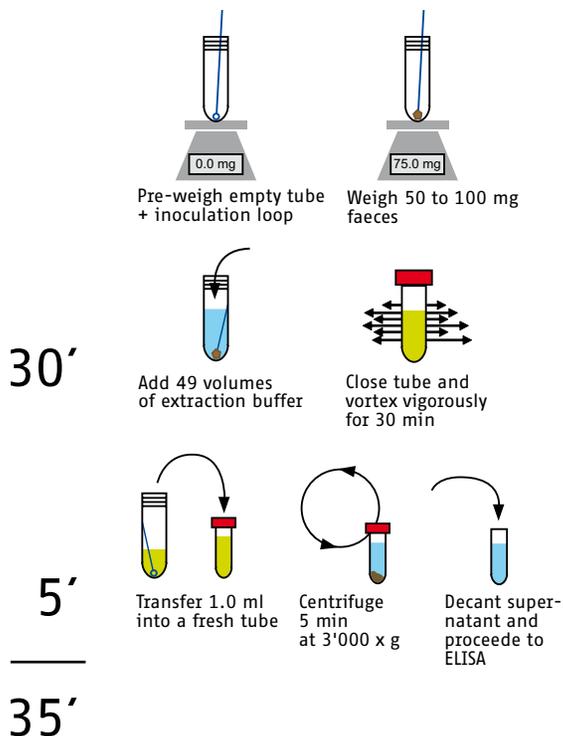## Extraction Methods

General comments

Homogenize well. Fibrous, insoluble material occasionally present in the sample and turbidity of the final extract do not affect results.

1. Manual extraction procedure

Pre-weigh empty tube + inoculation loop
Weigh 50 to 100 mg faeces

30′

Add 49 volumes of extraction buffer
Close tube and vortex vigorously for 30 min

5′

Transfer 1.0 ml into a fresh tube
Centrifuge 5 min at 3'000 x g
Decant supernatant and proceede to ELISA

35′

2. Extraction Devices

CALEX® Cap

15′

- Remove the white sampling pin from the tube by pulling it upwards.
- Insert the sampling pin 3-5 times into the sample and make sure that grooves are completely filled.
- Re-introduce the sampling pin into the CALEX® Cap tube and push it back into the final locking position.
- Vortex the CALEX® Cap device with the white head down vigorously on a vortex mixer for 30 sec and allow it to stand for 10 min on the white head. Repeat the procedure until the grooves are completely free of stool.
- Centrifuge it for 5 minutes at 500 x g and continue with the ELISA.

Smart-Prep

6′

- Press the sample into the hollow cavity in the base cap and level off the surface.
- Firmly press the tube onto the base cap and detach the tag.
- Add 4 mL of extraction buffer.
- Homogenize the sample for 1 min using a vibration mixer.
- Pour the extract into a fresh tube.
- Centrifuge it for 5 minutes at 3'000 x g and continue with the ELISA.

ELISA

All reagents must be adjusted to room temperature

Precoated Microtiter Plate

wash 2x

100 µL Calibrators, Controls or diluted Samples (1:50 or 1:150)

30′ incubate 30 min at 18-28°C on a plate rotator

wash 3 x

add 100 µL Enzyme Label

30′ incubate 30 min at 18-28°C on a plate rotator

wash 5 x

add 100 µL TMB Substrate

15′ incubate 15 min at 18-28°C on a plate rotator

add 100 µL Stop Solution

Read at 450 nm

75′

Time to Result: <2 hours

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BÜHLMANN fCAL® ELISA   Characteristics EK-CAL-U

Intended Use

Extraction and quantitative in vitro determination of human Calprotectin in stool samples. For Research Use Only. Not for use in diagnostics procedures in the US.

Pre-Analytics

Sample required:	50-100 mg human faeces
Sample collection:	plain tubes without chemical or biological additives
Sample storage	at 2-8°C up to 6 days
Storage of extracts:	at 2-8°C up to 7 days and at ≤-20°C at least 24 months

Special Equipment

- Multi tube vortex mixer
- Precision balance (10-150 mg)
- Fecal extraction device
  (to be ordered separately)
- Micro centrifuge

Calibrators & Controls

5	Calibrators*	lower range ELISA procedure: 10-600 µg/g
		extended range ELISA procedure: 30-1800 µg/g
2	Controls	low, high

* The standard sample dilution has already been factored in the calibrators!

Assay Performance Data

For lower and extended range ELISA procedure

Intra-assay precision **4.7 %**
n=6; range: 52.5-1246 µg/g
20 duplicates; CV range: 2.7-8.1 %

Inter-assay precision **<15 %**
n=10; range: 18.1-1764 µg/g
20 repetitions; CV range: 6.6-14.5 %

Dilution linearity **103 %**
12 samples diluted 1:50 up to 1:12800
recovery range: 82-124 %

Spiking recovery **100 %**
2 samples spiked with native Calprotectin from human serum; recovery range: 97-103 %

Analytical sensitivity **<10 µg/g[1]**
n=20 each , in duplicates; mean + 2 SD **<30 µg/g[2]**

Functional sensitivity **<10 µg/g[1]**
n=28; range: 5.2-2080 µg/g **<30 µg/g[2]**
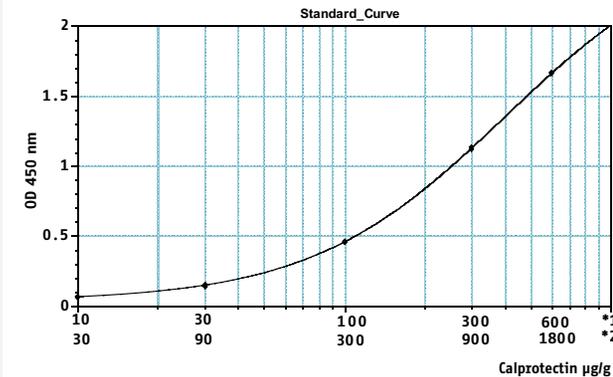Each 20 x in duplicates; limit of acceptance 15 % CV

Precision profile **<15 %**
The precision profile allows a precise measurement between 10-600 and 30-1800 µg/g.

[1] lower range ELISA procedure
[2] extended range ELISA procedure

Typical Standard Curve (4 Parameter Logistics)

Standard_Curve

Available For Research Use Only in the US.

US: BÜHLMANN fCAL® ELISA (EK-CAL-U, EK-CAL2-U, EK-CAL-WEX-U) and MRP8/14 ELISA (EK-MRP8/14-U) are available For Research Use Only in the US.

Canada: BÜHLMANN fCAL® ELISA (EK-CAL) is Health Canada Licensed (No. 80726).

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