



# BÜHLMANN fCAL<sup>®</sup> ELISA

Procedure

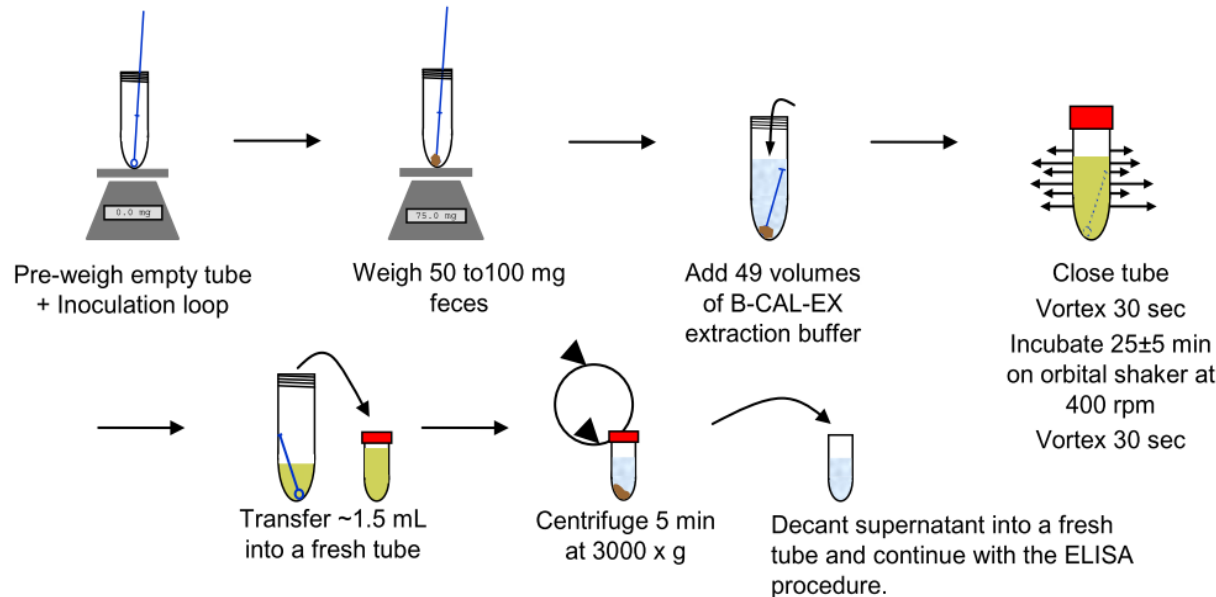
EK-CAL

## Extraction Method

### General comments

- To achieve quantitative results, it is important to completely homogenize the added stool sample in the extraction buffer. There may be a small amount of insoluble (undigested) particles remaining after mixing. The particles should not affect the results.
- Turbidity of the extract may still be observed after the 5 minute centrifugation step. The turbidity can be reduced by further centrifugation, but such turbidity has not been found to affect the quantitative results.

## Extraction procedure



## Sample Storage

### Specimen transport

Stool specimens should be received for processing by the laboratory within 3 days of collection. Stool specimens may be shipped at room temperature or on cold packs.

### Specimen storage

Stool specimens should be refrigerated at 2-8 °C and extracted within 3 days of receipt at the laboratory. Do not store samples at elevated temperatures.

### Extract storage

Fecal calprotectin extracts are stable refrigerated for up to 7 days at 2-8 °C. For longer storage, freeze the extracts at -20 °C. Frozen extracts are stable for a period of up to 36 months (3 years).

## ELISA

All reagents must be adjusted to room temperature

- Dilute Sample (1:150)
- Precoated Microtiter Plate

↓ wash 2x and blot

add 100 µl Incubation Buffer (blank) (duplicate), Calibrators (duplicate), Controls (duplicate) or diluted Samples (1:150)

30' ↓ Incubate 30 to 35 min at RT on a plate rotator ~450 rpm  
wash 3 x

add 100 µl Enzyme Label

30' ↓ Incubate 30±5 min at RT on a plate rotator ~450 rpm  
wash 5 x

add 100 µl TMB Substrate Solution

15' ↓ Incubate 15 min at RT on a plate rotator ~450 rpm

add 100 µl Stop Solution

→ Read at 450 nm

75' Time to Result: <2 hours



# BÜHLMANN fCAL® ELISA

## Characteristics EK-CAL

### Intended Use

The BÜHLMANN fCAL® ELISA is an in vitro diagnostic assay intended for the quantitative measurement of fecal calprotectin in human stool. The BÜHLMANN fCAL® ELISA aids in the diagnosis of inflammatory bowel disease (IBD), specifically Crohn's disease (CD) and ulcerative colitis (UC) and aids in the differentiation of IBD from irritable bowel syndrome (IBS) in conjunction with other laboratory and clinical findings.

### Pre-Analytics

- Sample required: 50-100 mg human feces
- Sample collection: plain tubes without chemical or biological additives

**Store the stool samples refrigerated at 2-8°C until ready for transport to the laboratory.**

### Calibrators & Controls

- 5 Calibrators\* ELISA range: 30-1800 µg/g
- 2 Controls low, high

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

### Assay Performance Data

**Within-laboratory precision** **3.1-9.4 %CV**  
Seven stool specimen extracts ranging from 38.5 to 918 µg/g  
10 days x 2 runs x 4 replicates

**Reproducibility** **6.4-23.2 %CV**  
Five stool specimen extracts ranging from 41.4 to 1056.6 µg/g  
3 routine laboratory sites x 5 days x 2 runs x 4 replicates using  
3 different reagent lots

**Dilution linearity** **24.6-2096.2 µg/g**  
Three experiments using dilution series with calprotectin levels covering the measuring range of the assay; correlation coefficients of the linear fits ranging from R<sup>2</sup>=0.994-0.998

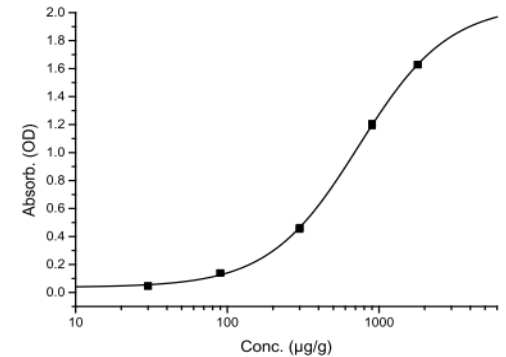
**Spiking recovery** **96.4-102.2%**  
Seven samples spiked with calibrator material (human serum based)

**Limit of Quantitation (LoQ)** **30 µg/g**  
n=60

**Limit of Detection (LoD)** **12.6 µg/g**  
n=60

**Limit of Blank (LoB)** **8.3 µg/g**  
n=60

### Typical Standard Curve (4 Parameter Logistics)



### Safety Precautions

- The microtiter strips, calibrators and controls of this kit contain components of human origin. Although tested and found negative for HBV surface antigen, as well as for HCV and HIV1/2 antibodies, these reagents should be handled as if capable of transmitting infections.
- The stop solution contains sulfuric acid. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection.

BÜHLMANN fCAL® is a registered trademark of BÜHLMANN in many countries.

**Ordering code:**  
EK-CAL 96 wells  
EK-CAL2 192 wells

FDA cleared 510(k) Nr. K181012



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