**BÜHLMANN fCAL® ELISA**

**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).

**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**

1. Dilute Sample (1:150)
2. Precoated Microtiter Plate  
   - wash 2x and blot
   - add 100 µl Incubation Buffer (blank) (duplicate), Calibrators (duplicate), Controls (duplicate) or diluted Samples (1:150)
3. Incubate 30 to 35 min at RT on a plate rotator ~450 rpm
4. wash 3 x
5. add 100 µl Enzyme Label
6. Incubate 30±5 min at RT on a plate rotator ~450 rpm
7. wash 5 x
8. add 100 µl TMB Substrate Solution
9. Incubate 15 min at RT on a plate rotator ~450 rpm
10. add 100 µl Stop Solution
11. Read at 450 nm

**ELISA**
All reagents must be adjusted to room temperature

**Time to Result:** <2 hours
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 R²=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: EX-CAL 96 wells EX-CAL2 192 wells
FDA cleared 510(k) Nr. K181012