BÜHLMANN fCAL® ELISA

Calprotectin ELISA

For *In Vitro* Diagnostic Use

EK-CAL	96 tests
EK-CAL2	192 tests

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Version A3

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ENGLISH

INTENDED USE

The BÜHLMANN fCAL® ELISA is an *in vitro* diagnostic test for the quantitative determination of calprotectin in human stool specimens intended as an aid to the assessment of intestinal mucosal inflammation. The assay results can be used as an aid to diagnosis in distinguishing organic, inflammatory disease of the gastrointestinal tract (inflammatory bowel disease, IBD, specifically Crohn's disease or ulcerative colitis, UC) from functional disease (irritable bowel syndrome, IBS) (ref. 1-7), in patients with chronic abdominal pain and as an aid to IBD disease monitoring (ref. 7-18).

For laboratory use only.

PRINCIPLE OF THE ASSAY

The BÜHLMANN fCAL® ELISA allows the selective measurement of calprotectin in fecal extracts by sandwich ELISA. The microtiter plate of the BÜHLMANN fCAL® ELISA is coated with a monoclonal capture antibody (mAb) highly specific to the calprotectin heterodimeric and polymeric complexes. Patient fecal sample extracts, controls for determination of ELISA run acceptability, and calibrators are loaded onto wells of the microtiter plate. After a 30-minute incubation at room temperature and washing steps, a detection antibody (Ab) conjugated to horseradish peroxidase (HRP) detects the calprotectin molecules bound to the capture antibody on the plate. After incubation and further washing steps, the chromogenic HRP substrate, tetramethylbenzidine (TMB) is added (blue color formation) followed by a stopping reaction (change to yellow color). The absorption is measured at 450 nm. The final calprotectin concentration in µg/g stool in the patient samples is determined using the calibration curve generated from the measured calibrator values.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity		Code	Reconsti-tution
	EK-CAL	EK-CAL2		
Extraction Buffer	3 bottles x 125 mL	6 bottles x 125 mL	B-CAL-EX	Ready to use
Microtiter Plate precoated with anti-calprotectin mAb	12 x 8 well strips with frame	2 x 12 x 8 well strips with frame	B-CAL-MP	Ready to use
Plate Sealer	3 pieces	6 pieces	-	Ready to use
Wash Buffer concentrate (10x) with preservatives	1 bottle x 100 mL	2 bottles x 100 mL	B-CAL-WB	Dilute each with 900 mL of deionized H_2O
Incubation Buffer with preservatives	2 bottles x 125 mL	3 bottles x 125 mL	B-CAL-IB	Ready to use
Calibrators A to E[1) 2)] Serum-derived calprotectin in a buffer matrix with preservatives	5 vials x 1 mL	5 vials x 1 mL	B-CAL-CASET	Ready to use
Control Low / High[3)] Serum-derived calprotectin in a buffer matrix with preservatives	2 vials x 1 mL	2 vials x 1 mL	B-CAL-CONSET	Ready to use
Enzyme Label Anti-calprotectin Ab conjugated to HRP	1 vial x 12 mL	2 vials x 12 mL	B-CAL-EL	Ready to use
TMB Substrate TMB in citrate buffer	1 vial x 12 mL	2 vials x 12 mL	B-TMB12	Ready to use
Stop Solution 0.25 M sulfuric acid	1 vial x 12 mL	2 vials x 12 mL	B-STS12	Ready to use **Corrosive agent**

Table 1

[1)] The actual calprotectin concentration of the calibrators A to E are 4, 12, 40, 120 and 240 ng/mL, respectively. For the lower range ELISA procedure the calibrator values have to be set as: 10, 30, 100, 300 and 600 µg/g calprotectin. This assignment corresponds to the final 1:2500 sample dilution in the lower range ELISA procedure.

[2)] If you choose the extended range ELISA procedure the nominal calibrator values have to be set as: 30, 90, 300, 900 and 1800 µg/g calprotectin. This assignment corresponds to the final 1:7500 sample dilution in the extended range ELISA procedure.

[3)] The controls contain lot specific amounts of native human calprotectin. Refer to the additional QC data sheet for actual concentrations.

STORAGE AND STABILITY OF REAGENTS AND WORKING SOLUTIONS

Sealed / unopened reagents	
Store at 2-8 °C. Do not use the reagents beyond the expiration date printed on the labels.	
Opened / reconstituted reagents	
Extraction Buffer	Store for up to 6 months at 2-8 °C.
Microtiter Plate	Return unused strips immediately to the foil pouch containing the desiccant packs and reseal along the entire edge of zip-seal. Store for up to 6 months at 2-8 °C.
Diluted Wash Buffer	Store for up to 6 months at 2-8 °C.
Incubation Buffer	
Calibrators	
Controls	
Enzyme Label	
TMB Substrate	
Stop Solution	

Table 2

REAGENTS AND MATERIALS SUPPLIED SUPPLEMENTARY

Fecal extraction devices

Fecal extraction devices described in table 3 are not delivered with the kit. The selected extraction devices must be ordered separately.

Extraction Device Kits	Quantity	Code
CALEX® Cap	Packages of 50, 200 or 500 tubes available, each filled with 5 mL extraction buffer Ready to use	B-CALEX-C50 B-CALEX-C200 B-CALEX-C500
Smart-Prep	50 tubes, spatulas, and base caps	B-CAL-RD

Table 3

MATERIALS REQUIRED BUT NOT PROVIDED

Extraction procedure

- 100 µL and 1000 µL precision pipettes with disposable tips
- Disposable polystyrene or polypropylene tubes for transfer of extracts (optional)
- Laminar flow workstation
- Multi tube vortex mixer / Vortex mixer
- Micro centrifuge (≥3000 x g)
- Centrifuge (≥500 x g)
- Extraction devices (see table 3 above) or for the manual extraction procedure:
  - 10 µL disposable inoculation loops
  - 15 mL polypropylene tubes with screw caps required
  - Precision balance (10-200 mg)

ELISA procedure

- 10 µL, 100 µL and 1000 µL precision pipettes with disposable tips
- Disposable polystyrene or polypropylene tubes for the preparation of sample dilutions
- 1000 mL cylinder for the dilution of the wash buffer
- Microtiter plate washer (see technical precautions) or squeeze bottle for wash buffer
- Microtiter plate shaker (see technical precautions)
- Blotting paper
- Microtiter plate reader for measurement of absorbance at 450 nm

WARNINGS AND PRECAUTIONS

Safety precautions

- The calibrators and controls of this test contain components of human origin. Although tested and found negative for HBV surface antigen, HCV and HIV1/2 antibodies, the reagents should be handled as if capable of transmitting infections and should be handled in accordance with Good Laboratory Practices (GLP) using appropriate precautions.
- This kit contains components classified in accordance with the Regulation (EC) No. 1272/2008:

  2-methyl-4-isothiazolin-3-one hydrochloride (conc. ≥ 0.0015%), thus the reagents may cause allergic skin reactions (H317).

  Sulfuric acid (conc. ≥2.5 - <5%), thus the reagents may cause skin irritation (H315) and serious eye irritation (H319.)
- Avoid contact of reagents with the skin, eyes or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, irritation / burns can occur.
- Reagents and chemicals have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

Technical precautions

Kit components

- Residues in the microtiter plate wells result from the production process. They are removed in the washing step (assay procedure step 3) and do not affect the results.
- Components must not be used after the expiry date printed on the labels.
- Do not mix or use components from kits with different lot numbers.
- Every effort should be made to ensure that no cross contamination occurs between reagents, samples or between wells.
- Allow the reagents to equilibrate to room temperature. Mix (vortex) the reagents well before use.
- Microwells cannot be re-used.

Manual extraction procedure

- To achieve quantitative results, it is important to completely homogenize the added stool sample in the extraction buffer. Contaminations at the top of the extraction tube should be avoided. There may be a small amount of insoluble particles remaining after mixing.

ELISA procedure

- In the ELISA procedure the washing steps are essential to guarantee reproducible results. Allow the wash buffer to incubate in the wells for a minimum of 20 seconds before removing.
- If an automated washer is used, “plate mode” is strongly recommended, i.e. each process step (dispense / aspiration) is carried out on all of the strips, sequentially, before the instrument continues with the next washing cycle. Thus, the minimal incubation time is guaranteed.
- The indicated number of washing cycles is mandatory to ensure reproducible results.
- The plate shaker must be adjusted to approximately 450 rpm (7.5 Hz). Higher rotation frequency may cause poor dilution linearity at values between 300/900 and 600/1800 µg/g. A rotational movement should be used instead of a horizontal movement.
- To ensure the antigen/antibody reaction is complete, the incubation time in step 5 must be at least 30 minutes. Moderately longer incubation time (up to 5 minutes) has no influence on the final outcome.
- The enzyme label is inactivated by oxygen and is highly sensitive to sodium azide, thimerosal, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Therefore, only deionized high quality water must be used.
- A new standard curve must be generated each time the assay is performed (each plate or partial plate).
- If the initial concentration of an unknown sample reads above the concentration of the highest calibrator, calibrator E, the sample may be further diluted with incubation buffer and assayed again according to the assay procedure. The resulting total dilution factor must be taken into account for the calculation of results.

SPECIMEN COLLECTION AND STORAGE

For the extraction procedure, less than 1 g of native stool specimen is required. Collect stool specimen into plain tubes.

Important: The specimen must be collected without any chemical or biological additives.

Specimen transport
Stool specimens should be received for processing by the laboratory within 3 days of collection. The specimens may be transported at room temperature or refrigerated.

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.

STOOL SAMPLE EXTRACTION AND EXTRACT STABILITY

1. CALEX® Cap

1.1 Extraction procedure

Follow the instruction for use provided with the CALEX® Cap device (Code B-CALEX-C50 / B-CALEX-C200 / B-CALEX-C500).

CALEX® Cap device: Liquid stool samples can be pipetted directly into the CALEX® Cap device. Unscrew the blue cap and pipet 10 µL of stool sample into the device. Recap the CALEX® Cap device and proceed with vortexing step according to the extraction procedure described and illustrated in the instruction for use delivered with the CALEX® Cap device.

Important: After extraction, centrifuge the CALEX® Cap device for 5 minutes at 500-3000 x g and continue with the assay procedure.

1.2 Extract storage

Fecal calprotectin extracts obtained with the CALEX® Cap device are stable at room temperature (23 °C) for 7 days and at 2-8 °C for up to 15 days. For longer storage, freeze extracts at -20°C. Frozen extracts are stable for a period of up to 23 months.
CALEX® Cap extracts can be frozen directly and stored within the CALEX® Cap device. Extracts can be subject to four freeze-thaw cycles. Prior to measurement, allow frozen extracts to equilibrate to room temperature, vortex thoroughly for 10 seconds and centrifuge for 5 minutes at 500-3000 x g.

2. Other extraction devices

2.1 Extraction procedures

Follow the instruction for use delivered with the respective extraction device:

- Fecal extraction device Roche (Code 10745804322) or BÜHLMANN Smart-Prep (Code: B-CAL-RD).

Important: After extraction with BÜHLMANN Smart Prep centrifuge the tubes for 5 minutes at 3000 x g. Alternatively, transfer the homogenate into a 2 mL Eppendorf tube and centrifuge it in a microcentrifuge for 5 minutes at 3000 x g. Decant the supernatant into a fresh, labeled tube and continue with the assay procedure.

2.2 Extract storage

Fecal calprotectin extracts obtained with Smart-Prep are stable at 2-8 °C for ≤7 days or at -20 °C for 36 months.

3. Manual extraction

3.1 Extraction procedure

1. Label and weigh the empty polypropylene tube including the inoculation loop. Note the weight (tare).
2. Take out 50 to 100 mg of the stool sample by means of the inoculation loop and place it into the pre-weighted tube and weigh it again (gross weight). Avoid taking up dietary fibers present in the sample during the sampling process.
3. Calculate the net amount of sample, by subtracting tare from the gross weight, break the inoculation loop away, and leave the lower part of the loop in the tube.
4. Add extraction buffer according to the formula
   x mg stool x 49 = y µL extraction buffer (e.g. 50 mg stool + 2450 µL buffer) to the tube and close the tube.
5. Extract the samples by
   - Vigorously vortex the extraction tube containing buffer and stool sample on a (multitube) vortexer (at highest speed) for 30 seconds.
   - Next, incubate the extraction tube for 25 +/- 5 minutes on a plate shaker at ca. 400 rpm. The inoculation loop inside the tube serves to enhance agitation.
   - Again, vigorously vortex the extraction tube for 30 seconds.
6. Transfer 1.5 mL of the homogenate into a 2 mL Eppendorf tube and centrifuge in a microcentrifuge for 5 minutes at 3000 x g.
7. Decant the supernatant into a fresh labeled tube and continue with the assay procedure or store extracts (refer to extract storage).

3.2 Extract storage

Fecal calprotectin extracts obtained by manual extraction, can be stored at 2-8 °C for ≤7 days or at -20 °C for 36 months.

WORKING RANGE

The assay can be performed according to the following procedures: lower or extended range ELISA procedure. The appropriate procedure should be selected depending on the expected calprotectin concentration:

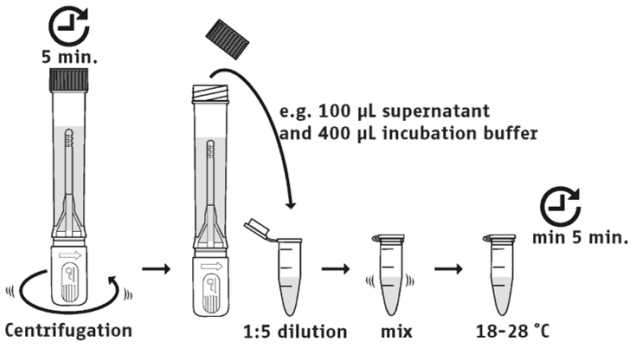
- For samples up to 600 µg/g calprotectin select lower range procedure (working range 10-600 µg/g).
- If the samples tend to exceed 600 µg/g select the extended range procedure (working range 30-1800 µg/g).

ASSAY PROCEDURE

Important: Allow all reagents to equilibrate for at least 30 minutes to 18-28 °C prior to use. Only dilute stool extracts. Calibrators and controls are ready to use.

1. Sample dilution option 1:
Working range 10-600 µg/g

1.1. CALEX® Cap device: Dilute the stool extracts 1:5 with incubation buffer (e.g. 100 µL extract and 400 µL incubation buffer) and mix well. Let the samples equilibrate for at least 5 minutes at 18-28 °C prior to proceeding to step 4c.

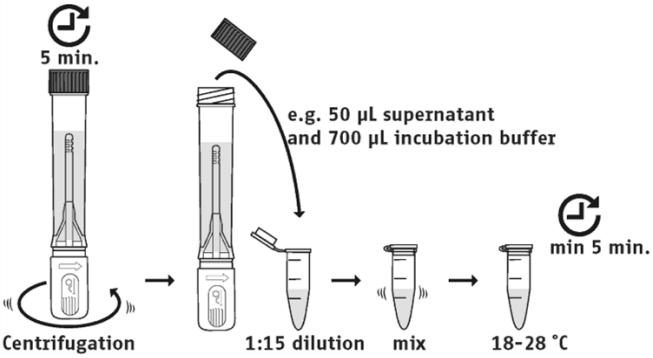


1.2 Manual extraction or Smart-Prep: Dilute the stool extracts 1:50 with incubation buffer (e.g. 20 µL extract and 980 µL incubation buffer) and mix well. Let the samples equilibrate for at least 5 minutes at 18-28 °C prior to proceeding to step 4c.

1.` Sample dilution option 2:
Working range 30-1800 µg/g

The working range can be extended by a factor of 3, if you dilute the samples 1:7500 instead of 1:2500. This procedure is recommended, if high calprotectin concentrations are to be expected.

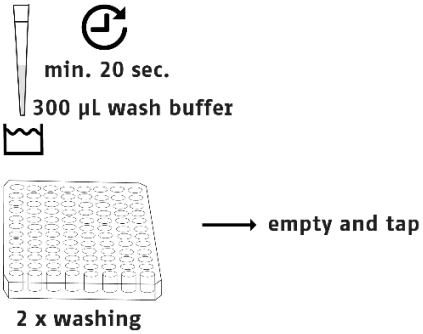
1.1` CALEX® Cap device: Dilute the stool extracts 1:15 with incubation buffer (e.g. 50 µL extract and 700 µL incubation buffer) and mix well. Let the samples equilibrate for at least 5 minutes at 18-28 °C prior to proceeding to step 4c.



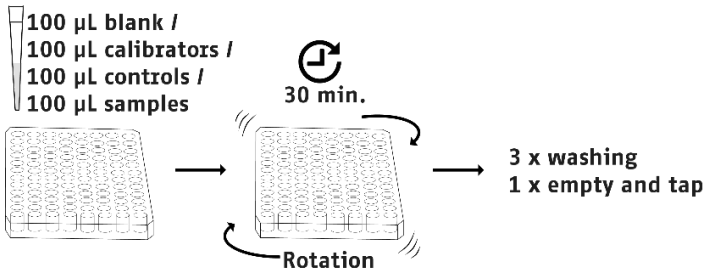
1.2` Manual extraction or Smart-Prep: Dilute the stool extracts 1:150 with incubation buffer (e.g. 20 µL extract and 2980 µL incubation buffer) and mix well. Let the samples equilibrate for at least 5 minutes at 18-28 °C prior to proceeding to step 4c.

2. Prepare a plate frame with sufficient strips to test the required number of calibrators, controls and diluted samples. Remove excess strips from the frame and re-seal them in the foil pouch together with the desiccant packs without delay. Store refrigerated.
3. Wash the coated wells twice using at least 300 µL of wash buffer per well. Empty the wells and tap the plate firmly onto blotting paper.

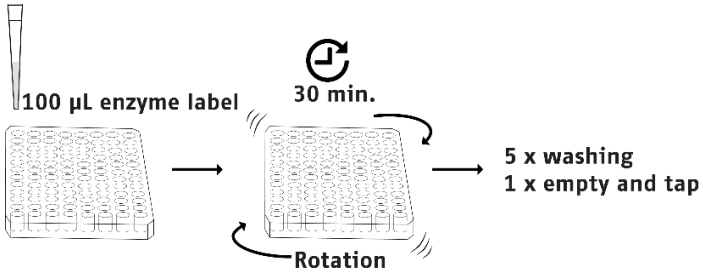
Important: Allow wash buffer to remain in the wells for a minimum of 20 seconds during each wash step.



4a. Pipet 100 µL of incubation buffer (blank) and
Pipet 100 µL of calibrator A-E into the respective wells.
4b. Pipet 100 µL of the controls low and high into the respective wells.
4c. Pipet 100 µL of each diluted sample into the subsequent wells.
5. Cover the plate with a plate sealer, and incubate for 30 + max. 5 min on a plate shaker set to ~450 rpm at 18-28 °C (see technical precautions – ELISA procedure).
6. Remove and discard the plate sealer. Empty the wells and wash three times using at least 300 µL of wash buffer per well (see technical precautions – ELISA procedure). Empty the wells and tap the plate firmly onto blotting paper.

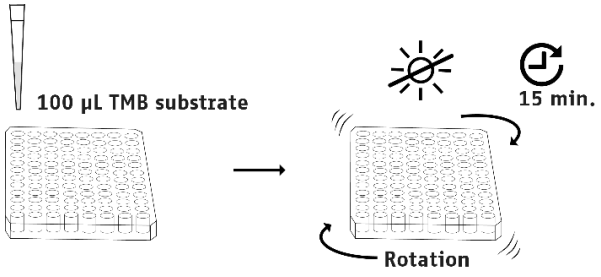


7. Pipet 100 µL of enzyme label to all wells.
8. Cover the plate with a plate sealer, and incubate for 30 ±5 min on a plate shaker set to ~450 rpm at 18-28 °C.
9. Remove and discard the plate sealer. Empty the wells and wash five times using at least 300 µL of wash buffer per well. Empty the wells and tap the plate firmly onto blotting paper.



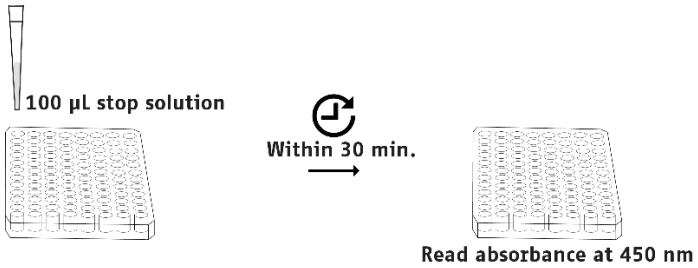
Important: Allow the TMB substrate solution to equilibrate to 18-28 °C.

10. Pipet 100 µL of the TMB substrate solution to all wells.
11. Cover the plate with a plate sealer, protect the plate from direct light and incubate for 15 ±2 min on a plate shaker set to ~450 rpm at 18-28 °C.



12. Pipet 100 µL of stop solution to all wells. Remove air bubbles with a pipette tip. Proceed to step 13 within 30 min.

13. Read the absorbance at 450 nm in a microtiter plate reader.



QUALITY CONTROL

Thorough understanding of this instruction for use is necessary for the successful use of the product. Reliable results will be obtained only by precise laboratory techniques and accurately following this instruction for use.

The BÜHLMANN fCAL® ELISA kit comes with two controls: controls low and high. The corresponding reference values of the controls are stated in the QC data sheet provided with each kit. The values and ranges stated on the QC data sheet always refer to the current kit lot and should be used for direct comparison of the results. Should the results for the controls low and/or high be out of the range stated in the QC data sheet, it is recommended to consider the whole run as invalid.

It is recommended to use internal control samples, in addition to kit controls, according to local and national regulations. The use of internal control samples is advised to assure the day to day validity of results. Since there is no control for fecal calprotectin commercially available, we recommend using a pool of stool extracts with normal and pathological levels for internal quality control.

The reproducibility of standard curve parameters and control values should be within established limits of laboratory acceptability. If the performance of the assay does not meet the established limits and repetition has excluded errors in technique, check the following issues: i) pipetting, temperature controlling and timing devices; ii) ELISA reader settings; iii) expiration dates of reagents; iv) storage and incubation conditions; v) TMB substrate solution should be colorless; vi) purity of water; vii) aspiration and washing methods.

STANDARDIZATION AND METROLOGICAL TRACEABILITY

There are no internationally or nationally recognized reference materials or reference measurement procedures for the calprotectin analyte in stool specimen. The BÜHLMANN fCAL® ELISA calibrator values are assigned in multiple measurement runs using internal reference material based on human serum and the BÜHLMANN fCAL® ELISA measurement procedure. The calprotectin concentration of the internal reference material was established using purified MRP8/14 from human granulocytes as primary reference material.
The 95% confidence interval of the combined uncertainty of product calibrators was determined as lower than 13.3%, the combined uncertainty of the product controls lower than 16.4%.

CALCULATION OF TEST RESULTS

Standard curve

It is recommended to use a software program capable of the following calculations; subtract the blank OD value from each calibrator well to calculate the calibrator value. Establish a standard curve using a 4-parameter logistic (4 PL) fit.

Controls and Samples

It is recommended to use a software program capable of the following calculations; subtract the blank OD value from each control/ sample well. Calculate the calprotectin concentration of the control/ sample in each well, in µg/g, using the established standard curve.

Working range 10-600 µg/g

If you select the lower range ELISA procedure, the calibrator concentrations have to be set as: 10, 30, 100, 300 and 600 µg/g calprotectin. Additional dilution factors (if using a different final dilution than 1:2500) have to be multiplied with the results to obtain the final results.

Refer to table 12 and figure 1 for typical data (results and standard curve). These results and standard curve are provided for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.

Working range 30-1800 µg/g

If you select the extended range ELISA procedure, the following nominal calibrator values have to be set as: 30, 90, 300, 900 and 1800 µg/g calprotectin. Additional dilution factors (if using a different final dilution than 1:7500) have to be multiplied with the results to obtain the final results.
Refer to table 15 and figure 3 for typical data (results and standard curve). These results and standard curve are provided for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.

LIMITATIONS

- Reagents delivered with the BÜHLMANN fCAL® ELISA kit are intended for the determination of calprotectin levels in human stool samples only.
- Test results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.
- For IBD disease monitoring, multiple fecal calprotectin measurements performed at up to 4 weeks intervals have been suggested to have best diagnostic accuracy in predicting clinical relapse in patients (ref. 19-20).
- Results may not be clinically applicable to children less than 4 years of age who have mildly increased fecal calprotectin levels (ref. 21-24).
- Some patients taking non-steroidal anti-inflammatory drugs (NSAID) will have elevations in their fecal calprotectin levels.

INTERPRETATION OF RESULTS

I. Distinguishing organic disease from functional gastrointestinal disease

The result categories are based on data from clinical studies performed by BÜHLMANN and are BÜHLMANN's

recommendations. All test results should be interpreted in conjunction with information available from the patient's clinical symptoms, medical history, and other clinical and laboratory findings.

Clinical thresholds

Results from 58 clinical samples from patients diagnosed with IBS and 131 clinical samples from patients diagnosed with IBD, from an international clinical study, were analyzed to obtain the values described in table 4.

Calprotectin concentration	Interpretation	Follow-up
< 80 µg/g	Normal	None
80 – 160 µg/g	Gray-zone/Borderline	Follow-up within 4 – 6 weeks
> 160 µg/g	Elevated	Repeat as needed

Table 4

Calprotectin values below 80 µg/g

Fecal calprotectin values <80 µg/g are not indicative of inflammation in the gastrointestinal tract. Patients with low calprotectin levels are not likely to be in need of invasive procedures to determine the inflammation cause.

Calprotectin values between and equal to 80 and 160 µg/g

Mid-fecal calprotectin levels between and equal to 80 and 160 µg/g, also called gray-zone levels, are not directly indicative of an active inflammation requiring immediate follow-up with invasive testing. However, the presence of inflammation cannot be excluded. Re-evaluation of fecal calprotectin levels after 4 to 6 weeks is recommended to determine the inflammatory status.

Calprotectin values above 160 µg/g

Fecal calprotectin values >160 µg/g are indicative of neutrophil infiltrate in the gastrointestinal tract; therefore, this may signal the presence of active inflammatory disease. Appropriate further investigative procedures by specialists are suggested to achieve an overall clinical diagnosis.

Clinical evaluation

The ability of the BÜHLMANN fCAL® ELISA to discriminate between patients with IBD and other non-inflammatory GI disorders, including IBS, was tested in a clinical study with a total of 337 adult and pediatric patients. One hundred and thirty five (135) patients had a final diagnosis of IBD (Crohn's disease, ulcerative colitis or indeterminate colitis), 130 patients suffered from IBS and 72 patients presented with abdominal pain and/or diarrhea, or other GI-related non-inflammatory conditions (refer to table 5). Final diagnosis was supported by endoscopic as well as other clinical findings.

A clinical sensitivity of 93.3% at 80 µg/g and a clinical specificity of 83.7% at 160 µg/g, can be reached in the differentiation between IBD and GI-related non-inflammatory conditions, including IBS. ROC curve analysis resulted in an AUC of 0.923 (refer to table 6).

A clinical sensitivity of 93.3% at 80 µg/g and a clinical specificity of 85.4% at 160 µg/g, can be reached in the differentiation between IBD and IBS. ROC curve analysis resulted in an AUC of 0.933 (refer to table 8).

The optimal cut-off combination for these patient pools could be defined by ROC analysis at 80 µg/g and 160 µg/g calprotectin, which is slightly more stringent than a combination of **a more sensitive lower cut-off of 50 µg/g** with lower performance in specificity, and **an upper cut-off of 200 µg/g** with slightly lower sensitivity (table 7 and 9).

II. IBD monitoring

Clinical thresholds and evaluation

The determination of fecal calprotectin is a reliable and simple way to assist the monitoring of IBD patients (ref. 7-18).

Correlation of calprotectin levels and the inflammatory status of patients' intestinal mucosa, according to endoscopic evaluations, were determined in three independent studies using BÜHLMANN calprotectin tests (table 10). The diagnostic value of calprotectin in predicting clinical remission and relapse, according to patient`s symptoms, clinical activity indices, unplanned need for therapy escalation, hospitalization or emergency was determined in three studies using BÜHLMANN calprotectin tests (table 11).

The result categories shown are recommendations and their establishment is based on condensed knowledge of published cut-offs and clinical performance studies. It is advised that healthcare practitioners establish individual patient thresholds by determining the patient's baseline calprotectin level during disease remission.

Calprotectin values below 100 µg/g

Fecal calprotectin levels below 100 µg/g can reliably indicate patients, with low risk of clinical relapse, in endoscopic remission for whom invasive endoscopic procedures can be avoided (ref. 7-18).

Calprotectin values between 100 and 300 µg/g

Fecal calprotectin levels between 100-300 µg/g may indicate the necessity of tighter control in the following period to assess disease development tendencies.

Calprotectin values above 300 µg/g

Fecal calprotectin levels above 300 µg/g should be repeated and, if raised levels are confirmed, prompt further investigative procedures (ref. 7-18).

PERFORMANCE CHARACTERISTICS

The performance characteristics of the BÜHLMANN fCAL® ELISA were established using the manual extraction method, unless otherwise stated.

Working range: 10 - 600 µg/g

Repeatability: 1.9 - 8.0% CV

Within-laboratory precision: 5.5 - 14.0% CV

Repeatability and within-laboratory precision were established based on the CLSI guideline EP05-A2 using a 22 days x 2 replicates study design. Ten extracted stool samples with calprotectin concentrations ranging from 13.2 - 501.4 µg/g were tested (table 13).

Limit of Detection (LoD): 4.2 µg/g

The LoD was established according to the CLSI guideline EP17-A and with proportions of false positives (α) less than 5% and false negatives (β) less than 5% based on 240 determinations, with 80 blank (extraction buffer) and 160 low level replicates; and a **LoB of 0.29 µg/g**.

Limit of Quantitation (LoQ): 9.8 µg/g

The LoQ was established using data obtained in the within

-laboratory precision study, including an additional stool sample with a concentration of 7.4 µg/g. The LoQ was determined as the calprotectin concentration at which the non-linear fit of total precision data intersected the precision goal of 20% CV.

Linearity: 10 - 600 µg/g

The linear range of the BÜHLMANN fCAL® ELISA was determined according to the CLSI guideline EP06-A. A maximum deviation from linearity of ±20% was allowed. For values below 75 µg/g an absolute difference of less than ±15 µg/g was allowed (table 14).

Accuracy/Recovery
Total bias: -1.1%;
Lower Limit of Agreement: -17.5%,
Upper Limit of Agreement: 15.4%

Four negative extracted stool samples were spiked with increasing amounts of calprotectin from serum specimens. The results are presented in figure 2.

High dose hook effect

Samples with theoretical concentrations of up to ~60'000 µg/g can be assayed without limiting the measuring range of the assay.

Working range: 30 - 1800 µg/g

Repeatability: 1.7 - 5.8% CV
Within-laboratory precision: 3.1 - 9.4% CV

Repeatability and within-laboratory precision were established according to the CLSI guideline EP05-A3 using a 10 days x 2 runs x 4 replicates study design. Seven pooled stool specimen extracts with calprotectin concentrations ranging from 38.5 - 918.0 µg/g were tested (table 16).

Between-lot precision: 4.2 - 9.7% CV

Between-lot precision was established according to the CLSI guideline EP05-A3 using a 3 lots x 5 days x 5 replicates study design and a random effects variance components model. Six stool specimen extracts with calprotectin concentrations ranging from 46.4 - 1476.1 µg/g were tested (table 17).

Reproducibility (Multisite precision evaluation study): 6.4 – 19.0% CV

Reproducibility was established according to the CLSI guideline EP05-A3 using a 3 laboratory sites x 2 operators x 5 days x 2 runs per day x 4 replicates study design. Three reagent lots were used in the study. Five pooled stool specimen extracts with calprotectin concentrations ranging from 42.1 - 1053.3 µg/g were tested (table 18).

Limit of Detection (LoD): 12.6 µg/g

The LoD was established according to the CLSI guideline EP17-A2 and with proportions of false positives (α) less than 5% and false negatives (β) less than 5% based on 120 determinations, with 60 blank (extraction buffer) and 60 low level replicates; and an **LoB of 8.3 µg/g**.

Limit of Quantitation (LoQ): 21.3 µg/g

The LoQ was established according to the CLSI guideline EP17-A2, based on 60 determinations and a precision goal of 20% CV.

Linearity: 30 - 1800 µg/g

The linear range of the BÜHLMANN fCAL® ELISA was determined according to the CLSI guideline EP06-A. A maximum deviation from linearity of ±20% was allowed. For values below 75 µg/g an absolute difference of less than ±15 µg/g was allowed (table 19).

Accuracy / Recovery: 96.4 – 102.2%

Seven stool specimen extracts with calprotectin levels ranging between 46.5 - 990.2 µg/g were spiked with 180 µg/g calprotectin in calibrator material. Spiking was performed at 10% the specimen extract volume. "Baseline" samples were spiked with the corresponding amount of incubation buffer. "Baseline" and "baseline + spike" samples were measured in three replicates (table 20).

Preanalytics

The performance characteristics of the BÜHLMANN fCAL® ELISA with regard to preanalytical procedures was established using the working range 30 – 1800 µg/g.

Extraction reproducibility – manual extraction: 9.5 - 20.5%

Extraction reproducibility was established according to the CLSI guideline EP05-A3 using a 10 extractions x 2 replicates study design. Nine clinical stool samples, including solid, semi-solid and liquid sample consistency, with calprotectin concentrations in the range of 51.2 - 1783.7 µg/g, were tested (table 21).

Extraction reproducibility – CALEX® Cap: 7.9 - 16.9%

Extraction reproducibility was established according to the CLSI guideline EP05-A3 using a 3 CALEX® Cap lots x 4 extractions x 4 replicates study design. Five clinical stool samples, including solid, semi-solid and liquid sample consistency, with calprotectin concentrations in the range of 42.5 - 2949.9 µg/g, were tested (table 22).

Method comparison CALEX® Cap vs. manual extraction
Bias at 80 µg/g: 5.9% (95% CI: 1.4 - 12.2%)
Bias at 160 µg/g: 12.0% (95% CI: 7.8 – 17.0%)
Mean Bias: 10.1% (95% CI: 5.7 – 14.5%)

The method comparison study was performed according to the CLSI guideline EP09-A3. Two hundred forty one (241) clinical samples were extracted using one lot of the CALEX® Cap device. Reference values, with a final calprotectin concentration interval of 30.5 - 1496.6 µg/g were established using the manual extraction method. Extracts were measured in single determinations in both methods. Bias was determined using Passing-Bablok linear regression and Bland-Altman analysis (tables 23 and 24).

INTERFERING SUBSTANCES

The susceptibility of the BÜHLMANN fCAL® ELISA assay to oral pharmaceuticals, nutritional supplements, hemoglobin as well as entheropathological micro-organisms was assessed according to the CLSI guideline EP07-A2, using the extended working range. Bias in results exceeding 10% was considered as interference. No interference was detected with substances, listed in table 25, up to the indicated concentrations. No interference was detected with entheropathological micro-organisms, listed in table 26, up to the indicated amounts of colony forming units (CFU) per mL of stool specimen extract.

TABLES AND FIGURES

Clinical studies

Clinical study – distinguishing organic disease from functional gastrointestinal disease

Final diagnosis	Distribution of patients' results in numbers (percent) within BÜHLMANN fCAL® ELISA diagnostic ranges.			
	< 80 µg/g	80 - 160 µg/g	> 160 µg/g	Total
IBD	9 (6.7%)	12 (8.9%)	114 (84.4%)	135 (100%)
IBS	94 (72.3%)	17 (13.1%)	19 (14.6%)	130 (100%)
Other GI	48 (66.7%)	10 (13.9%)	14 (19.4%)	72 (100%)

Table 5

IBD vs. non-IBD	Clinical decision point	
	80 µg/g	160 µg/g
Sensitivity (95% CI)	93.3% (87.7%, 96.9%)	84.4% (77.2%, 90.1%)
Specificity (95% CI)	70.3% (63.5%, 76.5%)	83.7% (77.8%, 88.5%)
PPV (95% CI)	67.7% (60.5%, 74.4%)	77.6% (69.9%, 84.0%)
NPV (95% CI)	94.0% (89.0%, 97.2%)	88.9% (83.6%, 93.0%)
ROC AUC (95% CI)	0.923 (0.893, 0.953)	

Table 6

IBD vs. non-IBD	Clinical decision point	
	50 µg/g	200 µg/g
Sensitivity (95% CI)	96.3% (91.6%, 98.8%)	80.7% (73.1%, 87.0%)
Specificity (95% CI)	59.9% (52.8%, 66.7%)	87.1% (81.7%, 91.4%)
PPV (95% CI)	61.6% (54.7%, 68.2%)	80.7% (73.1%, 87.0%)
NPV (95% CI)	96.0% (91.0%, 98.7%)	87.1% (81.7%, 91.4%)

Table 7

IBD vs. IBS	Clinical decision point	
	80 µg/g	160 µg/g
Sensitivity (95% CI)	93.3% (87.7%, 96.9%)	84.4% (77.2%, 90.1%)
Specificity (95% CI)	72.3% (63.8%, 79.8%)	85.4% (78.1%, 91.0%)
PPV (95% CI)	77.8% (70.6%, 83.9%)	85.7% (78.6%, 91.2%)
NPV (95% CI)	91.3% (84.1%, 95.9%)	84.1% (76.7%, 89.9%)
ROC AUC (95% CI)	0.933 (0.902, 0.963)	

Table 8

IBD vs. IBS	Clinical decision point	
	50 µg/g	200 µg/g
Sensitivity (95% CI)	96.3% (91.6%, 98.8%)	80.7% (73.1%, 87.0%)
Specificity (95% CI)	59.2% (50.3% ,67.8%)	90.0% (83.5%, 94.6%)
PPV (95% CI)	71.0% (63.9%, 77.5%)	89.3% (82.5%, 94.2%)
NPV (95% CI)	93.9% (86.3%, 98.0%)	81.8% (74.5%, 87.8%)

Table 9

non-IBD – IBS + other GI
CI – confidence interval
PPV – positive predictive value
NPV – negative predictive value
ROC AUC – area under receiver operating characteristic curve

Clinical studies – IBD monitoring

Calprotectin[1] vs IBD activity determined by endoscopic findings	Study 1 Spain (ref. 9)	Study 2 Spain (ref. 10)	Study 3 Australia, New Zealand (ref.11)
Patient number and demographics	89 (CD[2]) Ages: 32-58 44% male	123 (UC[3]) Ages: 18-85 66.4% male	99 (CD[2] after resection) Ages: 29-47 46.5% male
Cut-off	272 µg/g	280 µg/g	100 µg/g
NPV	98%	86%	91%
PPV	76%	80.3%	53%

Table 10

[1] Study 1 & 2 – Quantum Blue® fCAL and Quantum Blue® fCAL high range
Study 3 – BÜHLMANN fCAL® ELISA
[2] CD = Crohn`s disease patients
[3] UC = Ulcerative Colitis patients

Clinical studies – IBD monitoring

Calprotectin[1] vs future clinical remission or relapse	Study 4 UK (ref. 12)	Study 5 Spain (ref. 13)	Study 6 Spain (ref. 14)
Patient number and demographics	92 (CD[2]) 38% male	30 (CD[2]) adalimumab therapy Ages: 24-64 43.3% male	33 (CD[2]) 20 (UC[3]) infliximab therapy Ages: 18-68 47.2% male
Follow-up time after calprotectin measurement	12 months	4 months	12 months
Patients in clinical relapse after follow-up	11%	30%	23%
Cut-off	240 µg/g	204 µg/g	160 µg/g
NPV	96.8%	100%	96.1%
PPV	27.6%	75%	68.7%

Table 11

[1] Study 4 – BÜHLMANN fCAL® ELISA
Study 5 & 6 – Quantum Blue® fCAL and Quantum Blue® fCAL high range
[2] CD = Crohn`s disease patients
[3] UC = Ulcerative Colitis patients

TABLES AND FIGURES

LOWER RANGE PROCEDURE 10-600 µg/g

Example of results

	Conc. [µg/g]	Absorb. [OD]	Calc. Conc. [µg/g]	CV Conc [%]
Blank Avg.		**0.096**		
Cal A	10	0.073		
Cal A	10	0.066		
Cal A Avg.	**10**	**0.069**		**7.2**
Cal B	30	0.143		
Cal B	30	0.153		
Cal B Avg.	**30**	**0.148**		**4.8**
Cal C	100	0.465		
Cal C	100	0.456		
Cal C Avg.	**100**	**0.460**		**1.4**
Cal D	300	1.121		
Cal D	300	1.135		
Cal D Avg.	**300**	**1.128**		**0.9**
Cal E	600	1.658		
Cal E	600	1.671		
Cal E Avg.	**600**	**1.664**		**0.6**
Ctrl Low		0.201	41	
Ctrl Low		0.189	39	
Ctrl Low Avg.		**0.195**	**40**	**4.4**
Ctrl High		0.598	134	
Ctrl High		0.583	130	
Ctrl High Avg.		**0.590**	**132**	**1.8**

Table 12

Example of a standard curve

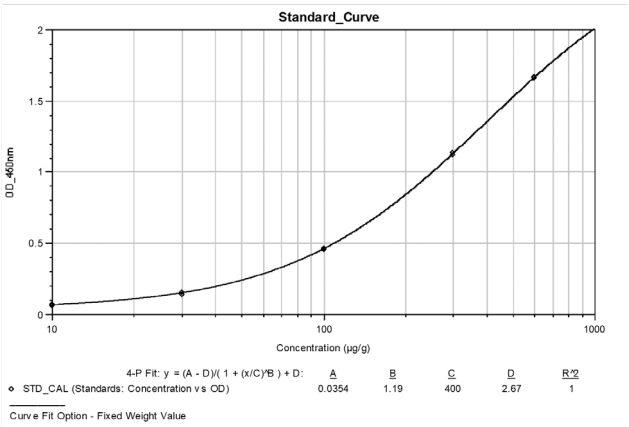


Figure 1

Within-laboratory precision

Sample No.	n	Mean [µg/g]	Repeatability		Between-day		Total Precision	
			SD	%CV	SD	%CV	SD	%CV
#1957	44	13.2	1.0	8.0%	1.5	11.6%	1.8	14.0%
#1933	42	20.5	0.9	4.2%	1.6	7.7%	1.8	8.8%
#1934	44	19.7	1.2	6.0%	1.6	8.4%	2.0	10.3%
#1935	44	37.1	1.2	3.2%	2.1	5.8%	2.4	6.7%
#1936	44	35.4	0.9	2.7%	2.5	7.4%	2.7	7.8%
#1937	44	58.6	1.6	2.9%	3.6	6.4%	3.9	7.0%
#1938	44	83.9	2.6	3.1%	4.3	5.2%	5.0	6.0%
#1939	44	141.4	2.6	1.9%	7.1	5.2%	7.5	5.5%
#1956	44	294.1	14.0	4.8%	18.0	6.2%	22.8	7.8%
#1940	44	501.4	27.7	5.7%	20.9	4.3%	34.7	7.1%

Table 13

Linearity

ID	Measuring range tested	R^2	p-value for non-linear coefficient	Linear range
S1	2.3 - 740.0	0.972	p >0.05	3.1 - 602.8
S2	5.1 - 999.5	0.988	p <0.05	5.1 – 654.0
S3	1.3 – 690.2	0.994	p <0.05	3.9 – 690.2
S4	9.6 – 827	0.940	p <0.05	9.6 – 658.7

Table 14

Spiking recovery

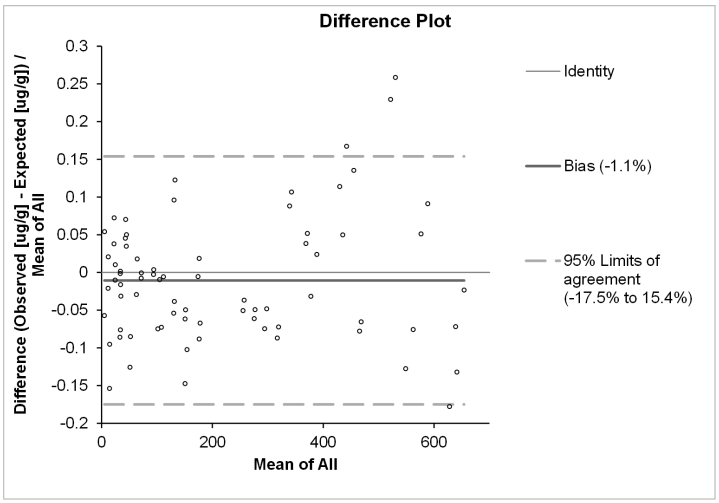


Figure 2

TABLES AND FIGURES

EXTENDED RANGE PROCEDURE 30-1800 µg/g

Example of results

	Concentration [µg/g]	Absorbtion [OD]
Calibrator A	30 30	0.047 0.046
Calibrator B	90 90	0.138 0.140
Calibrator C	300 300	0.464 0.452
Calibrator D	900 900	1.207 1.192
Calibrator E	1800 1800	1.627 1.630
Blank avg.		0.057
Control low		0.147 0.162
Control high		0.618 0.618

Table 15

Example of a standard curve

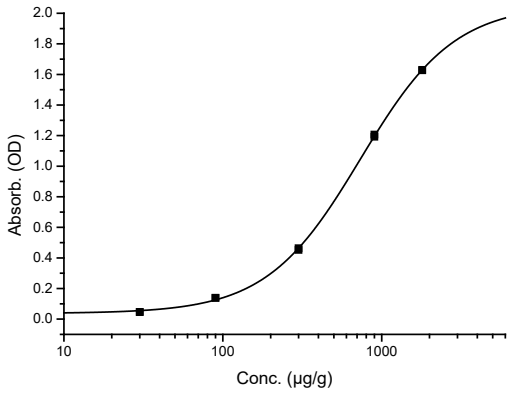


Figure 3

Within-laboratory precision

ID	Mean [µg/g]	n	Repeatability SD	Repeatability %CV	Between-run SD	Between-run %CV	Between-day SD	Between-day %CV	Within-laboratory SD	Within-laboratory %CV
P1	38.5	80	2.3	5.8%	1.8	4.8%	2.2	5.6%	3.6	9.4%
P2	67.0	80	2.0	3.0%	3.5	5.2%	1.6	2.4%	4.3	6.4%
P3	135.7	80	2.3	1.7%	5.6	4.1%	0.0	0.0%	6.0	4.4%
P4	207.1	80	4.1	2.0%	12.5	6.0%	0.0	0.0%	13.2	6.4%
P5	337.1	80	5.9	1.8%	18.3	5.4%	0.0	0.0%	19.3	5.7%
P6	562.6	80	11.0	2.0%	13.6	2.4%	2.5	0.4%	17.7	3.1%
P7	918.0	80	18.6	2.0%	62.1	6.8%	20.8	2.3%	68.1	7.4%

Table 16

Between-lot precision

ID	Mean [µg/g]	n	Within-run SD	Within-run %CV	Between-day SD	Between-day %CV	Between-lot SD	Between-lot %CV	Total SD	Total %CV
2	46.4	74	2.5	5.5%	0.5	1.1%	2.4	5.3%	4.5	9.7%
3	105.5	75	2.5	2.4%	1.4	1.4%	2.1	2.0%	4.5	4.2%
4	133.6	75	5.0	3.8%	1.9	1.4%	4.2	3.2%	7.2	5.4%
5	178.5	75	6.3	3.5%	0.0	0.0%	6.3	3.5%	9.2	5.2%
6	435.2	75	12.4	2.9%	7.5	1.7%	18.1	4.2%	23.2	5.3%
7	1476.1	75	48.4	3.3%	88.6	6.0%	31.4	2.1%	110.6	7.5%

Table 17

Reproducibility - Multisite Precision Evaluation Study

ID	Mean [µg/g]	n	Between-operator SD	Between-operator %CV	Between-site SD	Between-site %CV	Total (Reproducibility) SD	Total (Reproducibility) %CV
S01	42.1	236	0.0	0.0%	4.4	10.4%	8.0	19.0%
S02	67.4	238	1.7	2.6%	3.9	5.7%	8.6	12.7%
S03	142.3	238	2.4	1.7%	4.0	2.8%	16.8	11.8%
S04	379.8	240	0.0	0.0%	13.7	3.6%	24.2	6.4%
S05	1053.3	238	39.5	3.8%	64.4	6.1%	97.3	9.2%

Table 18

Linearity

ID	Measuring range tested [µg/g]	R^2	p-value for non-linear coefficient	Linear range [µg/g]
FRB	22.8 – 1932.0	0.998	$p < 0.05$	24.6 – 1932.0
FRC	26.2 – 2096.2	0.997	$p < 0.05$	26.2 – 2096.2

Table 19

Accuracy / Recovery

ID	Mean baseline [µg/g]	Expected baseline + spike [µg/g]	Observed baseline + spike [µg/g]	Recovery rate [%]
#1	46.5	226.5	224.5	99.1%
#2	63.7	243.7	247.7	101.6%
#3	89.0	269.0	274.9	102.2%
#4	111.6	291.6	292.0	100.1%
#5	163.5	343.5	331.1	96.4%
#6	304.0	484.0	475.0	98.1%
#7	990.2	1170.2	1166.6	99.7%

Table 20

TABLES AND FIGURES

PERFORMANCE CHARACTERISTICS - PREANALYTICS

Extraction reproducibility- manual weighing extraction

ID	Mean [µg/g]	n	Within-extraction		Between-extraction		Total precision	
			SD	%CV	SD	%CV	SD	%CV
S01	51.2	20	4.5	8.9%	7.8	15.2%	9.0	17.6%
S03	88.3	20	6.6	7.5%	13.3	15.0%	14.8	16.8%
S05	66.8	20	10.6	15.8%	3.7	5.5%	11.2	16.7%
S06	179.3	20	16.8	9.4%	32.8	18.3%	36.8	20.5%
S07	366.1	20	22.4	6.1%	32.3	8.8%	39.3	10.7%
S08	327.4	20	15.4	4.7%	26.9	8.2%	31.0	9.5%
S09	1783.7	20	198.3	11.1%	262.0	14.7%	328.6	18.4%

Table 21

Extraction reproducibility – CALEX® Cap

ID	Mean [µg/g]	Within-extraction		Between-extraction		Between -Lot		Total precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
A	42.5	1.6	3.9%	5.2	12.1%	0.0	0.0%	5.4	12.7%
B	126.5	3.2	2.5%	9.6	7.6%	9.3	7.4%	13.8	10.9%
C	207.4	5.1	2.4%	34.8	16.8%	0.0	0.0%	35.1	16.9%
D	515.5	13.9	2.7%	38.2	7.4%	0.0	0.0%	40.7	7.9%
E	2949.9	93.0	3.2%	214.6	7.3%	47.0	1.6%	238.6	8.1%

Table 22

Method comparison – CALEX® Cap extraction and manual extraction

Bland-Altman Analysis		
Mean Bias (95%)	Lower LoA (95% CI)	Upper LoA (95% CI)
10.1% (5.7%, 14.5%)	-47.4% (-54.9%, -39.8%)	67.5% (60.1%, 75.1%)

Table 23

Passing-Bablok Regression Analysis				
Slope (95% CI)	Intercept (µg/g) (95% CI)	Bias at 80 µg/g (95% CI)	Bias at 160 µg/g (95% CI)	r
1.181 (1.120, 1.235)	-9.7 (-16.0, -2.4)	5.9% (1.4%, 12.2%)	12.0% (7.8%,16.9%)	0.948

Table 24

INTERFERING SUBSTANCES

Oral pharmaceuticals, nutritional supplements and hemoglobin

Trade Name	Active Component	Concentration mg/50 mg stool
gyno-Tardyferon	Iron (II) sulfate (contains 0.35 mg folic acid)	0.11
Prednisone	Prednisone	0.31
Imurek	Azathioprine	0.19
Salofalk	Mesalamine; 5-ASA	5.21
Agopton	Lansoprazole	0.18
Asacol	Mesalamine; 5-ASA	2.50
Vancocin	Vancomycin	2.00
Sulfamethoxazole	Sulfamethoxazole	1.60
Trimethoprim	Trimethoprim lactate	0.35
Ciproxine	Ciprofloxacin	1.25
Vitamin E	DL-α-Tocopherol Acetate	0.30
Bion 3	3 probiotics (107 CFU): *lactobacillus gasseri* PA16 / 8, *bifidobacterium bifidum* MF 20/5, *bifidobacterium longum* SP07 / 3, 12 vitamins: A (800 µg), B1 (1.4 mg), B2 (1.6 mg), B6 (2 mg), B12 (1 µg), C (60 mg), D (5 µg), E (10 mg), Biotin (150 µg), folic acid (200 µg), niacin (18 mg), pantothenic acid (6 mg) and 7 minerals: iodine (100 µg), iron (5 mg), zinc (5 mg), selenium (30 µg), chromium (25 µg), manganese (1.2 mg), molybdenum (25 µg)	1.06
Hemoglobin	Hemoglobin	1.25

Table 25

Enteropathological microorganisms

Name	Final Concentration (CFU/mL stool extract)
Escherichia coli	9.5×10^7
Salmonella enterica subsp. enterica	1×10^9
Klebsiella pneumoniae subsp. pneumonia	5.4×10^7
Citrobacter freundii	9.7×10^7
Shigella flexneri	1.5×10^8
Yersinia enterocolitica subsp. enterocolitica	1.6×10^8

Table 26

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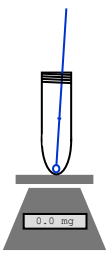
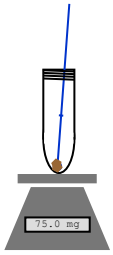
SHORT PROTOCOL

CALPROTECTIN EXTRACTION

Manual extraction procedure

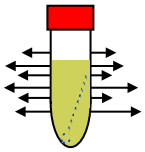


Pre-weigh empty tube
+ Inoculation loop

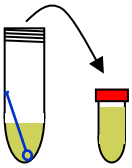


Weigh 50 to 100 mg feces

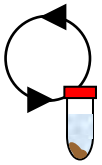
Add 49 volumes of B-CAL-EX extraction buffer



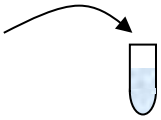
Close tube
Vortex 30 sec
Incubate 25±5 min on a shaker at 400 rpm
Vortex 30 sec



Transfer ~1.5 mL into a fresh tube



Centrifuge 5 min at 3000 x g



Decant supernatant into a fresh tube and continue with the ELISA procedure.

SHORT PROTOCOL

CALPROTECTIN ELISA

Precoated microtiter plate

↓

wash 2x with ≥300 µL wash buffer

100 µL incubation buffer, calibrators, controls or diluted samples

↓

incubate 30 (+ 5) minutes at 18-28 °C on a plate shaker ~450 rpm

wash 3x with ≥300 µL wash buffer

add 100 µL enzyme label

↓

incubate 30 +/-5 minutes at 18-28 °C on a plate shaker ~450 rpm

wash 5x *with ≥300 µL wash buffer*

add 100 µL TMB substrate solution

↓

incubate 15 +/-2 minutes at 18-28 °C on a plate shaker ~450 rpm

add 100 µL stop solution

→ **Read absorbance at 450 nm (within 30 minutes)**

TIME TO RESULT: 75 MINUTES

CHANGELOG

Date	Version	Change
2022-11-16	A3	Update to chapter *warnings and precautions* Inclusion of calibrator uncertainty values and justification of internal standardization in the chapter *Standardization* Wording update and simplification to chapter *Performance Characteristics* Revision of chapter *Symbols* New patent information Inclusion of notified body number to CE-mark – conformity assessment procedure according to IVDR 2017/746

INCIDENT REPORTING IN EU MEMBER STATES

If any serious incident in relation to this device has occurred, please report without delay to the manufacturer and competent authority of your Member State.

SHIPPING DAMAGE

Please notify your distributor, if this product was received damaged.

SYMBOLS

BÜHLMANN use symbols and signs listed and described in ISO 15223-1. In addition, the following symbols and signs are used:

Symbol	Explanation
MP	Microtiter plate
BUF EX	Extraction Buffer
BUF WASH 10X	Wash buffer concentrate (10x)
BUF INC	Incubation buffer
CAL A - CAL E	Calibrator A - E
CONTROL L	Control low
CONTROL H	Control High
EL	Enzyme label
SUBS TMB	TMB substrate
SOLN STOP	Stop solution
	**EN**: electronic instruction for use available in different languages at:/ **BG**: електронни инструкции за употреба на различни езици на адрес:/ **CS**: elektronický návod k použití dostupný v různých jazycích na adrese:/ **DA**: elektronisk brugsanvisning på forskellige sprog på:/ **DE**: elektronische Gebrauchsanweisung in verschiedenen Sprachen verfügbar unter:/ **EL**: ηλεκτρονικές οδηγίες χρήσης διαθέσιμες σε διάφορες γλώσσες στη διεύθυνση:/ **ES**: instrucciones de uso electrónicas disponibles en diferentes idiomas en:/ **ET**: elektrooniline kasutusjuhend, mis on saadaval erinevates keeltes aadressil:/ **FR**: un mode d'emploi électronique disponible en différentes langues à l'adresse:/ **HU**: különböző nyelveken elérhető elektronikus használati utasítás a következő címen:/ **IT**: istruzioni elettroniche per l'uso disponibili in diverse lingue su:/ **LT**: elektroninės naudojimo instrukcijos įvairiomis kalbomis:/ **LV**: dažādās valodās pieejama elektroniska lietošanas instrukcija:/ **NO**: elektronisk instruksjon for bruk tilgjengelig på forskjellige språk på:/ **PL**: elektroniczna instrukcja obsługi dostępna w różnych językach na stronie:/ **PT**: instrução electrónica para utilização disponível em diferentes línguas em:/ **RO**: instrucțiuni electronice de utilizare disponibile în diferite limbi la adresa:/ **SK**: elektronický návod na použitie dostupný v rôznych jazykoch na:/ **SL**: elektronska navodila za uporabo so na voljo v različnih jezikih na:/ **SR**: elektronsko uputstvo za upotrebu dostupno na različitim jezicima na:/ **SV**: elektronisk bruksanvisning på olika språk på följande adress: **www.buhlmannlabs.ch/support/downloads/**

Parts of the kit are patent protected by EP2947459(B1); US10620216(B2); AU2015261919(B2); JP6467436(B2).

CE 0123