Quantum Blue®
Calprotectin Extended

Quantitative
Lateral Flow Assay

For Research Use Only.
Not for use in diagnostic procedures.

LF-CALE25-U 25 tests

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ENGLISH

INTENDED USE

BÜHLMANN Quantum Blue® Calprotectin Extended assay is an immunoassay designed for the quantitative determination of Calprotectin concentrations in human stool samples (ref.1-6) in combination with the BÜHLMANN Quantum Blue® Reader.
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PRINCIPLE OF THE ASSAY

The test is designed for the selective measurement of Calprotectin antigen by sandwich immunoassay. A monoclonal capture antibody (mAb) being highly specific for Calprotectin is coated onto the test membrane. A second monoclonal detection antibody conjugated to gold colloids is deposited onto the conjugate release pad and released into the reaction system after addition of the extracted and diluted stool sample. The Calprotectin/anti-Calprotectin gold conjugate binds to the anti-Calprotectin antibody coated on the test membrane (test line; test band) and the remaining free anti-Calprotectin gold conjugate binds to the goat anti-mouse antibody coated on the test membrane (control line; control band). The signal intensities of the test line and the control line are measured quantitatively by the BÜHLMANN Quantum Blue® Reader.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Comments
Test Cassette	25 pieces	B-LFCALUS-TC	vacuum-sealed in a foil bag
Extraction buffer	1 bottle 125 mL	B-CAL-EX	Ready to use
RFID Chip Card	1 piece	B-CALE-RCC	**White** plastic card
RFID Chip Card	1 piece	B-CALE-RCC720	**Green** plastic card

Table 1

REAGENTS & MATERIAL SUPPLIED SUPPLEMENTARY

Fecal Extraction Devices

Fecal Extraction Devices described below are not delivered with the kit and either of them **has to be ordered** with the kit.

CALEX® Cap Device	50 or 200 tubes filled with 5 mL extraction buffer, ready to use	B-CALEX-C50-U B-CALEX-C200-U
Smart-Prep	50 tubes consisting of spatulas and base caps	B-CAL-RD-U
ScheBo® Quick-Prep™	50 tubes consisting of tube, cone & dosing tip. Filled with 1.3 mL extraction buffer, ready to use	B-CAL-SO50-U

Table 2

Controls

Controls for BÜHLMANN Quantum Blue® LF-CALE25-U can be ordered separately.

Controls Low* / High*	2 vials, 0.5 mL	B-CALE-CONSET-U	Ready to use

Table 3

* Lot specific concentration of Calprotectin

STORAGE AND SHELF LIFE OF REAGENTS

All kit components are stable at 2-8°C until the expiration date printed on the labels.

PRECAUTIONS

SAFETY PRECAUTIONS

- None of the reagents of this test contains components of human origin.
- Patient specimens should be handled as if capable of transmitting infections and should be handled in accordance with Good Laboratory Practice (GLP) using appropriate precautions.
- Unused solution should be disposed of according to local State and Federal regulations.

TECHNICAL PRECAUTIONS

Kit components

- All reagents and test samples must be equilibrated to room temperature (18-28°C) before starting the assay. Mix well (vortex) the reagents before use.
- Components must not be used after the expiry date printed on the labels.
- Do not mix different lots of reagents.
- Test Cassettes cannot be re-used.

Test Procedure

- Read carefully the instructions prior to carrying out the test. Test performance will be adversely affected, if reagents are incorrectly diluted, handled or stored under conditions other than those as detailed in this instruction for use.
- The Quantum Blue® Reader must be switched on and programmed for the Quantum Blue® Calprotectin assay: B-CALE-RCC or B-CALE-RCC720, before starting the assay (see Quantum Blue® Reader Manual).
- Use the RFID Chip Card in order to change lot-specific test parameters.
- Patient samples that are not properly handled may cause inaccurate results.
- In order to receive reliable and quantitative results it is important to homogenize the stool sample entirely in the Extraction Device.
- Diluted samples should be used within several hours and cannot be stored for a longer time period.
- With BÜHLMANN Smart Prep it is important to centrifuge the extracts before storage. Centrifuge the tubes for 5 minutes at 3'000 x g. After centrifugation the supernatant must be transferred into a fresh storage tube. With CALEX® Cap Device you can store the extracts directly with the device. For re-use/ re-measurement of the extracts see step 2 under the chapter Assay Procedure.

MATERIALS REQUIRED BUT NOT PROVIDED

- Vortex mixer for stool extraction
- Precision pipettes with disposable tips: 10-200 µl and 1 ml
- Centrifuge
- 5 ml polypropylene or polystyrene tubes for dilution of the extracts
- Timer (optional)
- Quantum Blue® Reader available from BÜHLMANN (order code: BI-POCTR-ABS)
- Soft tissues or blotting paper

SPECIMEN COLLECTION AND STORAGE

If the Extraction Devices are used (refer to Table 2), less than 1 g of native stool will be required.
Collect stool samples into plain tubes. The samples can be stored refrigerated at 2-8°C for at least 6 days.
The extracts are stable for at least 7 days at 2-8ºC and for at least 24 months at ≤ -20ºC.
Important: The sample must be collected without any chemical or biological additives in the collection device.

ASSAY PROCEDURE

The Assay Procedure consists of three steps:

1. **Extraction of stool samples:**
   The extraction is described in the instruction for use delivered with the respective extraction devices.
2. **Sample processing:**

- Smart Prep: After extraction, let the stool extract settle for 10 minutes. Dilute the supernatant 1:10 with extraction buffer (e.g. 50 µL extract and 450 µL extraction buffer) and mix well. Let the samples equilibrate for at least 5 minutes at 18-28°C prior to proceeding to the next step (step no. 3).
- CALEX® Cap Device: After extraction, let the stool extract settle for 10 minutes with the white head of the device down. Unscrew the blue cap. The supernatant can be used without further dilution in the lateral flow assay.

3. **Lateral Flow Assay Procedure and Readout:**
   Two alternative methods can be loaded from the respective RFID Chip card: B-CALE-RCC or B-CALE-RCC720. Select one of the RFID chip cards before starting the experiments. Load the test method from the RFID Chip Card.

3.1. Method <B-CALE-RCC720> with internal timer

- Use the green plastic card.
- Load the test cassette onto the test cassette holder of the Reader.
- Add 60 µl of diluted stool extract onto the sample loading port of the test cassette.
- Close the cassette holder and start the measurement by pressing the start button.
- The scan starts automatically after 12 minutes (720 seconds).

3.2. Method <B-CALE-RCC> without internal timer

- Use the white plastic card.
- Add 60 µl of diluted stool extract onto the sample loading port of the test cassette.
- Incubate for 12 minutes +/- 1 minute (set a timer manually).
- Load the test cassette onto the test cassette holder of the Reader.
- Scan the cassette with the Quantum Blue® Reader by pressing the start (<ENTER>) button immediately.

Remark: Please refer to the Quantum Blue® Reader Manual to learn about the basic functions and how to initialize and operate the Reader, especially how to select test methods, and how to load lot specific parameters from the RFID Chip Card in order to get the samples measured.

QUALITY CONTROL

- If the precision of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues: i) pipetting, temperature controlling and timing devices ii) expiration dates of reagents and iii) storage and incubation conditions.
- Result of the self-test of the Quantum Blue® Reader performed at startup of the instrument has to be valid.

VALIDATION OF RESULTS

- For a valid test result, the Control Line (C) must be visible in any case (see Figures 1A and 1B). It is used as functional test control only and cannot be used for the interpretation of the Test Line (T). If the Test Line (T) is not detectable after 12 minutes of incubation time (Figure 1A), the concentration of Calprotectin present in the stool sample is below the detection limit. If a Test Line (T) is detectable after 12 minutes of incubation time (Figure 1B), the Calprotectin concentration present in the stool sample is calculated by the Quantum Blue® Reader.
- If only the Test Line (T) is detectable after 12 minutes of incubation time (Figure 1C), the test result is invalid and the Calprotectin assay has to be repeated using another test Cassette.
- If neither the Control Line (C) nor the Test Line (T) are detectable after 12 minutes of incubation time (Figure 1D), the test result is invalid and the Calprotectin assay has to be repeated using another test Cassette.
- As the Quantum Blue® Reader allows a quantitative evaluation of the Test (T) and Control (C) Lines, an additional validity check of the Control Line (C) is undertaken. If the signal intensity of the Control Line (C) is below a threshold after 12 minutes of incubation time, the test result is also invalid and the Calprotectin High Range assay has to be repeated using another test Cassette.

STANDARDIZATION AND INTERPRETATION OF RESULTS

- The Lateral Flow Assay is calibrated with the BÜHLMANN fCAL™ ELISA (order code: EK-CAL-U).
- The BÜHLMANN Quantum Blue® Reader uses a lot-specific standard curve to calculate the Calprotectin concentration. The assay range is between 30 and 1000 µg/g.
- For quantitative measurements, unknown samples reading above 1000 µg/g can be re-tested in the BÜHLMANN Quantum Blue® Calprotectin High Range assay (order code: LF-CHR25-U).

LIMITATIONS

- The reagents supplied with this kit are optimized to measure human Calprotectin in extracted stool samples.

PERFORMANCE CHARACTERISTICS

Limit of Blank (LoB): <20 µg/g calprotectin**.**

Limit of Detection (LoD): <30 µg/g calprotectin.

Limit of Quantification (LoQ):
Lower LoQ: ≤30 µg/g calprotectin
Upper LoQ: ≥1000 µg/g calprotectin.
The LoQ has been established with 14 stool samples. The resulting precision profile is shown in Figure 2. The limit of quantification corresponds to the concentration of Calprotectin with an imprecision below 25% CV allowing a quantitative measurement within the range from 30 (lower LoQ) to 1000 µg/g (upper LoQ).

Linearity: The results showed linearity within the indicated measuring range of 30 to 1000 µg/g of the Quantum Blue® Calprotectin Extended assay for all samples.

Recovery: The recovery varied between 90.6% - 101.8%.

Repeatability: **20.1% mean CV.** The repeatability was calculated from 8 stool extract samples. The mean values of three different lots of Test Cassettes are presented in (Table 4). The repeatability varied between 17.9% -23.9 % CV.

Inter-lot Precision: 20.8% mean CV. The inter-lot precision of the Quantum Blue® Calprotectin Extended assay was calculated from 8 stool extract samples containing between 30 and 1000 µg/g calprotectin. The inter-lot data of the samples varied from 16.1% to 24.1% CV.

Method Comparison: R^2 = 0.94; y=1.05-3.91 µg/g
50 stool samples exhibiting calprotectin concentrations within the indicated measuring range of the Quantum Blue® Calprotectin Extended assay were analyzed according to the assay procedure and compared with the values obtained by the BÜHLMANN fCAL™ (order code: EK-CAL-U). The correlation data are illustrated in Figure 3.

Figure 1 **Test Results**

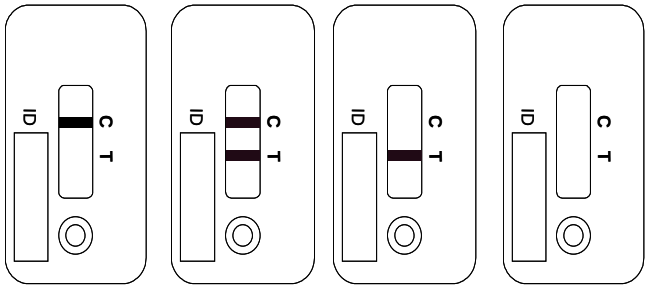


Figure 1A Figure 1B Figure 1C Figure 1D

Figure 2 **Precision Profile**

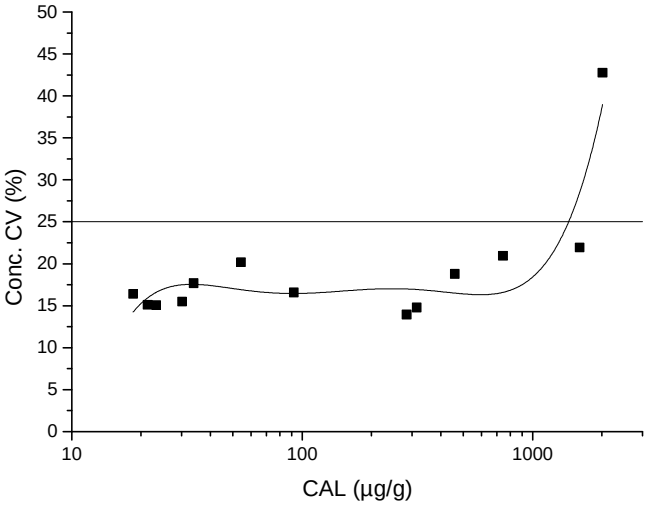
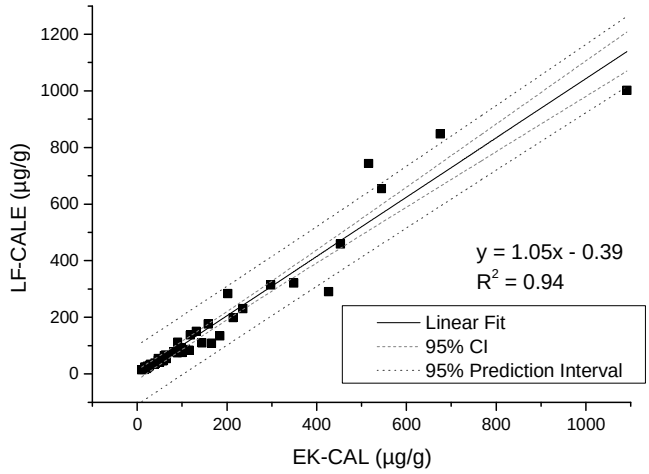


Table 4 **Precision (Repeatability)**

Sample No.	Calprotectin Concentration [mean µg/g]	Repeatability [CV]
Smpl 1	24.2	23.5%
Smpl 2	32.9	18.7%
Smpl 3	51.0	17.9%
Smpl 4	93.7	18.3%
Smpl 5	298.7	19.7%
Smpl 6	305.9	18.2%
Smpl 7	509.7	23.9%
Smpl 8	662.1	20.6%

Figure 3 **Method Comparison**



1. Tibble JA et al.: *Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease*. Gastroenterol **123**, 450-460 (2002)
2. Fagerhol MK: *Calprotectin, a faecal marker of organic gastrointestinal abnormality.* Lancet **356**, 1783-4 (2000)
3. Tibble JA *et al*.: *A simple method for assessing intestinal inflammation in Crohn's disease.* Gut **47**,506-513 (2000).
4. Tschanguizi et al.: *Stool Calprotectin as a Marker of inflammation.* Poster at UEGW, 2007
5. Hessian PA and Fisher L: *The heterodimeric complex of MRP-8 (S100A8) and MRP-14 (S100A9). Antibody recognition, epitope definition and the implications for structure*. Eur J Biochem 268, 353-63 (2001).
6. Konikoff MR and Denson LA: *Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease.* Inflamm Bowel Dis **12(6)**, 524-34 (2006)

Symbol	Explanation
	Use By
	Catalogue number
	Batch code
	Temperature limitation
	Content sufficient for <n> tests

Symbol	Explanation
	Consult Instructions for Use-
	Test Cassette
	Extraction Buffer
	RFID Chip card