# FCAL

BÜHLMANN

**fCAL turbo**

**Order information**

| REF | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|
| 08910367190 | fCAL turbo (200 tests) | System-ID 2149 001 | **cobas c** 303, **cobas c** 503 |

Materials required (but not provided):

| | | | |
|---|---|---|---|
| 08910499190 | fCAL Calibrator Set<br>fCAL Calibrator 1 (1 x 1 mL)<br>fCAL Calibrator 2 (1 x 1 mL)<br>fCAL Calibrator 3 (1 x 1 mL)<br>fCAL Calibrator 4 (1 x 1 mL)<br>fCAL Calibrator 5 (1 x 1 mL)<br>fCAL Calibrator 6 (1 x 1 mL) | <br>Code 20719<br>Code 20720<br>Code 20721<br>Code 20722<br>Code 20723<br>Code 20724 | |
| 08910502190 | fCAL Control Set<br>fCAL Control Level I (3 x 1 mL)<br>fCAL Control Level II (3 x 1 mL) | <br>Code 20167<br>Code 20168 | |
| 08910987001 | CALEX® Cap (500 pcs) | | |

**English**

**For use in the USA only**

**Roche does not hold the product registration for Partner Channels. The legal manufacturer indicated on the kit is solely responsible for all of the design, legal, and regulatory aspects of the product.**

For *in vitro* diagnostic use.

**Rx only**

**CLIA Complexity: High**

**System information**

**FCAL:** ACN 21490

**Intended use**

The fCAL turbo is an *in vitro* diagnostic assay intended for the quantitative measurement of fecal calprotectin, a neutrophilic protein that is a marker of intestinal mucosal inflammation, in human stool. fCAL turbo 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.

**Dedicated instruments**

**cobas c** 303 analyzers, **cobas c** 503 analyzers

**Summary**

Gastroenterologists are often faced with the diagnostic difficulty of differentiating individuals with functional gastrointestinal disorders, such as irritable bowel syndrome (IBS), from those with inflammatory bowel disease (IBD). Many symptoms are common to both conditions, whereas other clinical features such as a predominance of diarrhea and rectal bleeding will increase the likelihood of inflammatory disease. The clinical differentiation between these conditions remains problematic and may result in delayed diagnosis. Furthermore, many individuals with IBS must undergo invasive procedures (endoscopy) to rule out an organic disorder. This has significant implications for health care costs as well as exposing individuals to the inherent risks associated with invasive procedures.[1,2,3,4] Diseases included in the IBD category include Crohn´s disease (CD), ulcerative colitis (UC) and indeterminate colitis. IBD represents chronic and often disabling lifelong inflammatory conditions – frequently diagnosed in young people in their late teens and early twenties. It is estimated that nearly 1.2 million Americans are living with IBD, and the prevalence is rising.[5] The main difference between CD and UC is the location and nature of the inflammatory condition. In UC, the disease is restricted to the colon, whereas in CD, inflammation may affect any part of the gastrointestinal tract – the ileocecal area being most often affected.[6,7] The most striking difference between IBS and IBD is that the former is non-inflammatory in nature. Therefore, one possibility is to measure surrogate markers of intestinal inflammation to differentiate between the two.[8,9] Calprotectin is a calcium-binding protein found in neutrophilic granulocytes, monocytes, and macrophages, comprises up to 60 % of the total cytosolic protein content of neutrophils, resists metabolic degradation, and can be measured in feces.[10,11,12] Its use as a biomarker of intestinal inflammation has been extensively validated, showing consistently abnormal levels in the stool of individuals with IBD.[13,14,15,16]

**Test principle**

The fCAL turbo test is a particle-enhanced turbidimetric immunoassay (PETIA), which allows for automated quantification of calprotectin in fecal extracts on **cobas c** systems. Fecal samples are extracted with extraction buffer using the CALEX® Cap extraction device and applied at a final dilution of 1:500. The extracts are incubated with reaction buffer and mixed with polystyrene nanoparticles coated with calprotectin-specific antibodies (immunoparticles). Calprotectin available in the sample mediates immunoparticle agglutination. Sample turbidity, measured by light absorbance, increases with calprotectin-immunoparticle complex formation and is proportional to calprotectin concentration. The detected light absorbance allows quantification of calprotectin concentration via interpolation on an established calibration curve.

**Reagents - working solutions**

| Reagents | Quantity | Preparation |
|---|---|---|
| Reaction Buffer (R1)<br>MOPS buffered saline | 25.4 mL | Ready to use |
| Immunoparticles (R3)<br>Polystyrene beads coated with avian antibodies against human calprotectin | 6.5 mL | Ready to use |

R1 is in position B and R3 is in position C. R3 refers to the reagent, which is pipetted at time point R3.

**Precautions and warnings**

For *in vitro* diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Product safety labeling follows GHS guidance.

Contact phone: 1-800-428-2336

- This kit contains 2-methyl-4-isothiazolin-3-one hydrochloride (conc. ≥ 0.0015 %), thus the reagents may cause allergic skin reactions (H317).
- The immunoparticles contain potentially infectious substances of animal origin and should be handled in accordance with good laboratory practice (GLP) using appropriate precautions.
- R1 contains MOPS (3-(N-morpholino)propanesulfonic acid) (< 1 %), that can be irritating to eyes and skin. Handle with due caution.
- 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.
- Immunoparticles R3 contains polystyrene nanoparticles.
- Reagent immunoparticles R3, once frozen, cannot be used anymore. Freezing R3 will lead to reduced sensitivity and precision in low-level samples and in the worst case to decreased measurement levels.
- The assay is designed for fecal extract samples prepared using the

specific CALEX® Cap. Application of other extraction buffers could lead to incorrect results.
• Please equilibrate reagents, controls, calibrators and samples as described in this method sheet.
• Ensure that samples have no bubbles prior to running the test.
• Evaporation of calibrators and controls on the analyzer could lead to incorrect results. Run the assay immediately after loading the analyzer.

**Reagent handling**

Ready for use

**Loading of reagents**

The reagents supplied are ready to use. Mix gently before loading onto the instrument. Avoid bubble formation.

**Storage and stability**

*fCAL turbo*

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | store for up to 12 weeks at 5-15 °C |

**Do not freeze reagents!**

**Specimen collection and preparation**

The fCAL turbo is designed for calprotectin quantification in fecal sample extracts. Fecal samples are collected, extracted and diluted to a final concentration of 1:500 using the CALEX® Cap.

**Specimen transport and storage**

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

Follow the instruction for use provided with the CALEX® Cap. Fecal sample extracts prepared using the CALEX® Cap will have a final dilution of 1:500 and are ready to use. Liquid stool samples can be pipetted directly into the CALEX® Cap. Unscrew the blue cap and pipet 10 µL of stool sample into the device. Recap the CALEX® Cap and proceed with vortexing step according to the extraction procedure described and illustrated in the instruction for use delivered with the CALEX® Cap.

*Important:* Centrifuge the CALEX® Cap for 10 minutes at 1000-3000 g prior to running the fCAL turbo procedure.

CALEX® Cap extracts can be kept at room temperature for up to 2 hours, and after centrifugation, at 2-8 °C for up to 3.5 days (84 hours). For longer storage, freeze CALEX® Cap extracts at -20 °C. CALEX® Cap extracts can be subjected to 4 freeze-thaw cycles. Allow frozen extracts to equilibrate to room temperature for up to 2 hours before measurement. Prior to measurement, CALEX® Cap extract should be vortexed thoroughly for 10 seconds and centrifuged for 10 minutes at 1000-3000 g.

Please note that extracts can be stored and frozen directly within the CALEX® Cap.

**Materials provided**

See “Reagents – working solutions” section for reagents.

**Materials required (but not provided)**

- See “Order information” section for reference numbers
- General laboratory equipment
- fCAL Calibrator Set
- fCAL Control Set
- CALEX® Cap

**Assay**

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

**Application for fecal extracts**

**Test definition**

| | | |
|---|---|---|
| Reaction time | 10 min | |
| Wavelength (sub/main) | 800/546 nm | |
| Reagent pipetting | | Diluent ($H_2O$) |
| R1 | 100 µL | – |
| R3 | 16 µL | – |

| *Sample volumes* | *Sample* | *Sample dilution* | |
|---|---|---|---|
| | | Sample | Diluent ($H_2O$) |
| Normal | 8 µL | - | - |
| Decreased | 8 µL | 8 µL | 72 µL |
| Increased | 8 µL | - | - |

NOTE:
For technical reasons, it is necessary to specify a dummy unit (µg/mL) on **cobas c** analyzers. Values can be converted to the correct unit (µg/g) via the host computer.

**Calibration**

| | |
|---|---|
| Calibrators | S1: fCAL Calibrator 1 |
| | S2: fCAL Calibrator 2 |
| | S3: fCAL Calibrator 3 |
| | S4: fCAL Calibrator 4 |
| | S5: fCAL Calibrator 5 |
| | S6: fCAL Calibrator 6 |

Apply the lot-specific fCAL Calibrator values for the 6-point calibration curve:

| | |
|---|---|
| Calibration mode | Spline |
| Calibration frequency | Full calibration<br>• after reagent lot change<br>• after 9 weeks on-board the analyzer<br>• after 9 weeks when using a single reagent lot<br>• as required following quality control procedures |

To generate a reproducible calibration curve, equilibrate reagents R1 and R3 to the storage temperature of the analyzer by loading the **cobas c** pack at least 1 hour before calibration curve establishment. Keep calibrators as well as controls and patient samples at room temperature for at least 30 min before starting analysis. The calibration on **cobas c** 303 analyzers and **cobas c** 503 analyzers is performed in duplicate.

Traceability: This method has been standardized against internal reference material.

**Quality control**

For quality control, use control materials as listed in the "Order information" section.

**Validating instrument accuracy after calibration**

Run the fCAL Control Set, Level I and Level II, in duplicate, after each new calibration, before running patient fecal sample extracts. Keep controls at room temperature for at least 30 min before starting analysis.

**Validating daily runs**

Run the fCAL Control Set, Level I and Level II, each day, before running patient fecal sample extracts to validate calibration curve stability. Single measurements of controls are sufficient.

The controls have assigned target values indicated on the control value sheet supplied with each lot of the fCAL Control Set. All control measurements must be within target values indicated on the control value sheet to obtain valid results for patient fecal sample extracts. If the control values are not valid, repeat quality control measurement with fresh controls. If control values remain invalid, recalibrate the instrument. If valid control values cannot be reproduced after performing the steps described above, contact Roche US Technical Support 1-800-428-2336.

### Measuring patient samples

Once a calibration curve is established and validated with the quality controls, patient fecal extracts may be measured. Allow patient fecal sample extracts to equilibrate to room temperature for at least 30 minutes before starting analysis. Perform patient fecal extract measurement according to this instruction for use and instrument manual.

### Results

The results are evaluated automatically by the analyzer. The results of the fCAL turbo indicate the amount of calprotectin in µg per g of stool sample. For technical reasons, the results will be reported in the dummy unit of µg/mL and can be converted into µg/g via host computer by applying a factor of 1.0.

### Limits and ranges

The measuring range for the fCAL turbo assay on the **cobas c** 303 analyzers and **cobas c** 503 analyzers is 30-2000 µg/g. Samples > 2000 µg/g will be diluted automatically (1:10) by the analyzer, further extending the range to 30-10000 µg/g.

### Interpretation of results (clinical thresholds)

| 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 | repeats as needed |

Table 1: fCAL turbo diagnostic ranges

*Calprotectin values < 80 µg/g*
Fecal calprotectin values < 80 µg/g are not indicative of active inflammation in the gastrointestinal tract. Low fecal calprotectin levels can be used in conjunction with the patient's clinical symptoms, medical history and other clinical and laboratory findings to determine the need for additional diagnostic work-up. Specifically, for patients with a clinical and laboratory presentation suggesting a non-inflammatory disorder such as IBS, fecal calprotectin values of < 80 µg/g can be used to support a decision to defer invasive testing.

*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 4-6 weeks is recommended to determine the inflammatory status. This decision should be made by the clinician in conjunction with the patient's clinical symptoms, medical history and other clinical and laboratory findings.

*Calprotectin values > 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. Elevated fecal calprotectin levels can be used in conjunction with the patient's clinical symptoms, medical history and other clinical and laboratory findings to determine the need for further investigative procedures, including invasive procedures performed by specialists, to achieve an overall clinical diagnosis, in particular of IBD.

### Clinical evaluation

The clinical evaluation of fCAL turbo was assessed on the master system **cobas c** 501 analyzer. The results are also considered applicable to **cobas c** 303 analyzers and **cobas c** 503 analyzers.

The ability of the fCAL turbo to discriminate between patients with IBD and other non-inflammatory gastrointestinal (GI) disorders, including IBS, was evaluated using clinical samples collected from 337 adult and pediatric patients. One hundred and thirty five 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. Final diagnosis was supported by endoscopic as well as other clinical findings.

| Final diagnosis | Distribution of patients results in numbers (%) within fCAL turbo diagnostic ranges | | | |
|---|---|---|---|---|
| | < 80 µg/g | 80-160 µg/g | > 160 µg/g | Total |
| IBD | 12 (8.9 %) | 15 (11.1 %) | 108 (80 %) | 135 (100 %) |
| IBS | 99 (76.2 %) | 15 (11.5 %) | 16 (12.3 %) | 130 (100 %) |
| Other GI | 51 (70.8 %) | 7 (9.7 %) | 14 (19.4 %) | 72 (100 %) |

Table 2: Distributions of patients results within fCAL turbo diagnostic ranges

| IBD vs. non-IBD | Clinical decision point | |
|---|---|---|
| | 80 µg/g | 160 µg/g |
| Sensitivity (95 % CI) | 91.1 % (85.0 %, 95.3 %) | 80.0 % (72.3 %, 86.4 %) |
| Specificity (95 % CI) | 74.3 % (67.7 %, 80.1 %) | 85.1 % (79.5 %, 89.8 %) |
| PPV (95 % CI) | 70.3 % (62.9 %, 76.9 %) | 78.3 % (70.4 %, 84.8 %) |
| NPV (95 % CI) | 92.6 % (87.4 %, 96.1 %) | 86.4 % (80.9 %, 90.9 %) |
| ROC AUC (95 % CI) | 0.916 (0.884, 0.947) | |

Table 3: Clinical performance characteristics of the fCAL turbo in discriminating IBD from non-IBD – IBS and other GI-related disorders, at 80 µg/g and 160 µg/g clinical decision points

| IBD vs. IBS | Clinical decision point | |
|---|---|---|
| | 80 µg/g | 160 µg/g |
| Sensitivity (95 % CI) | 91.1 % (85.0 %, 95.3 %) | 80.0 % (72.3 %, 86.4 %) |
| Specificity (95 % CI) | 76.2 % (67.9 %, 83.2 %) | 87.7 % (80.8 %, 92.8 %) |
| PPV (95 % CI) | 79.9 % (72.7 %, 85.9 %) | 87.1 % (79.9 %, 92.4 %) |
| NPV (95 % CI) | 89.2 % (81.9 %, 94.3 %) | 80.9 % (73.4 %, 87.0 %) |
| ROC AUC (95 % CI) | 0.929 (0.898, 0.960) | |

Table 4: Clinical performance characteristics of the fCAL turbo in discriminating IBD from IBS at 80 µg/g and 160 µg/g clinical decision points

CI – confidence interval
PPV – positive predictive value
NPV – negative predictive value
ROC AUC – area under receiver operating characteristic curve

### Reference range

Stool samples were obtained from 141 apparently healthy normal adults (> 21 years of age) with no symptoms or signs of gastrointestinal disease. The test results were categorized by the assay cut-offs.

| | Distribution of results within fCAL turbo diagnostic ranges | | | |
|---|---|---|---|---|
| | < 80 µg/g | 80-160 µg/g | > 160 µg/g | Total |
| Number of subjects (%) | 106 (75.2 %) | 18 (12.8 %) | 17 (12.1 %) | 141 (100 %) |

Table 5: Distribution of healthy subjects results within fCAL turbo diagnostic ranges

### Specific performance data

The presented performance characteristics have been established on a **cobas c** 303 analyzer and **cobas c** 503 analyzer unless otherwise indicated, and should be considered as representative data. Results obtained in individual laboratories may differ.
For regulatory purposes, validation was performed using extracts obtained by manual weighing and extraction. The CALEX® Cap was validated for comparability with the manual extraction method on a **cobas c** 501 analyzer. Any changes to the directions for use, including special wash programming, may alter the stated performance characteristics of the assay and must be validated prior to implementation by the laboratory.

### Method comparison

The method comparison study was performed according to the CLSI guideline EP09-A3. Forty-five (45) clinical samples were measured on a **cobas c** 303 analyzer and **cobas c** 503 analyzer using 1 lot of fCAL turbo over 1 day in 1 calibration cycle. Reference values, with a final calprotectin concentration interval of 39.1-9075.4 µg/g, were established with the fCAL turbo on a **cobas c** 501 analyzer. Bias was determined using Passing-Bablok linear regression and Bland-Altman analysis.

| Bland-Altman analysis | | | Passing-Bablok regression analysis | | | | |
|---|---|---|---|---|---|---|---|
| Mean bias (95 % CI) | Lower LoA (95 % CI) | Upper LoA (95 % CI) | Slope (95 % CI) | y-Intercept (95 % CI) | Bias at 80 µg/g (95 % CI) | Bias at 160 µg/g (95 % CI) | r |
| 6.4 % (4.8 %, 7.9 %) | -3.7 % (-6.3 %, -1.0 %) | 16.4 % (13.7 %, 19.0 %) | 1.075 (1.049, 1.105) | -1.122 µg/g (-5.722 µg/g, 4.137 µg/g) | 6.1 % (1.5 %, 10.7 %) | 6.8 % (4.6 %, 8.7 %) | 1.000 |

Table 6: Summary of Bland-Altman and Passing-Bablok regression analyses obtained on **cobas c** 303 analyzer vs. established reference values on **cobas c** 501 analyzer. (LoA = Limits of agreement)

| Bland-Altman analysis | | | Passing-Bablok regression analysis | | | | |
|---|---|---|---|---|---|---|---|
| Mean bias (95 % CI) | Lower LoA (95 % CI) | Upper LoA (95 % CI) | Slope (95 % CI) | y-Intercept (95 % CI) | Bias at 80 µg/g (95 % CI) | Bias at 160 µg/g (95 % CI) | r |
| -7.0 % (-9.1 %, -4.8 %) | -20.9 % (-24.6 %, -17.3 %) | 7.0 % (3.3 %, 10.7 %) | 0.9866 (0.9657, 1.005) | -7.28 µg/g (-12.18 µg/g, -5.32 µg/g) | -10.4 % (-15.8 %, -9.2 %) | -5.9 % (-8.5 %, 4.9 %) | 0.998 |

Table 7: Summary of Bland-Altman and Passing-Bablok regression analyses obtained on **cobas c** 503 analyzer vs. established reference values on **cobas c** 501 analyzer. (LoA = Limits of agreement)

### Within-laboratory precision

Repeatability and within-laboratory precision were established according to the CLSI guideline EP05-A3. Six pooled stool specimen extracts with calprotectin concentrations covering the measuring range of the test and clinical decision points were tested over 5 days, in 2 runs per day, with 4 results generated per run. One reagent lot was used in the study.

| ID | Mean [µg/g] | Within-run (Repeatability) | | Between-run | | Between-day | | Total Precision | |
|---|---|---|---|---|---|---|---|---|---|
| | | SD [µg/g] | CV [%] | SD [µg/g] | CV [%] | SD [µg/g] | CV [%] | SD [µg/g] | CV [%] |
| P1 | 59.3 | 1.8 | 3.0 | 1.8 | 0.9 | 0.6 | 1.0 | 1.92 | 3.2 |
| P2 | 85.4 | 1.9 | 2.2 | 2.7 | 3.1 | 0.0 | 0.0 | 3.25 | 3.8 |
| P3 | 184.1 | 1.8 | 1.0 | 0.9 | 0.5 | 2.8 | 1.5 | 3.44 | 1.9 |
| P4 | 733.9 | 1.9 | 0.3 | 20.9 | 2.8 | 39.0 | 5.3 | 44.27 | 6.0 |
| P5 | 1738.4 | 5.7 | 0.3 | 37.1 | 2.1 | 36.0 | 2.1 | 52.00 | 3.0 |
| P6 | 6758.5 | 20.2 | 0.3 | 86.0 | 1.3 | 242.8 | 3.6 | 258.31 | 3.8 |

Table 8: Within-laboratory precision study results on the **cobas c** 303 analyzer

| ID | Mean [µg/g] | Within-run (Repeatability) | | Between-run | | Between-day | | Total Precision | |
|---|---|---|---|---|---|---|---|---|---|
| | | SD [µg/g] | CV [%] | SD [µg/g] | CV [%] | SD [µg/g] | CV [%] | SD [µg/g] | CV [%] |
| P1 | 49.0 | 1.8 | 3.6 | 1.5 | 3.2 | 1.6 | 3.2 | 2.8 | 5.7 |
| P2 | 64.5 | 1.8 | 2.7 | 1.6 | 2.4 | 4.3 | 6.7 | 4.9 | 7.6 |
| P3 | 163.4 | 1.3 | 0.8 | 2.6 | 1.6 | 4.3 | 2.6 | 5.2 | 3.2 |
| P4 | 735.2 | 1.8 | 0.2 | 10.6 | 1.4 | 27.4 | 3.7 | 29.4 | 4.0 |
| P5 | 1743.0 | 6.6 | 0.4 | 29.2 | 1.7 | 75.1 | 4.3 | 80.8 | 4.6 |
| P6 | 6657.9 | 27.2 | 0.4 | 105.3 | 1.6 | 0.0 | 0.0 | 108.7 | 1.6 |

Table 9: Within-laboratory precision study results on the **cobas c** 503 analyzer

### Extraction reproducibility-CALEX® Cap: 8.1-19.7 % CV

The extraction reproducibility was established according to the CLSI guideline EP05-A3 using a 2 days x 2 operators x 3 CALEX® Cap lots x 2 extractions x 3 replicates study design on the **cobas c** 501 analyzer. Twelve clinical stool specimens, including specimens with solid, semi-solid and liquid consistency, with calprotectin concentrations in the range of 42.7-3440.0 µg/g, were tested.

### Sample carry-over

The sample carry-over was established according to the CLSI guideline EP10-A2. No statistically significant sample carry-over with the fCAL turbo test on **cobas c** 303 analyzers and **cobas c** 503 analyzers was detected.

### Limit of Blank (LoB)

**cobas c** 303 analyzer: 9.8 µg/g

**cobas c** 503 analyzer: 2.6 µg/g

The LoB was established according to the CLSI guideline EP17-A2 with 4 negative (extraction buffer) samples. The samples were measured over 3 days in 5 replicates each day to produce 60 blank values. The LoB was calculated using non-parametric analysis. The study was performed with 1 reagent lot.

### Limit of Detection (LoD)

**cobas c** 303 analyzer: 12.9 µg/g

**cobas c** 503 analyzer: 6.3 µg/g

The LoD was established according to the CLSI guideline EP17-A2 with 4 stool specimen extracts with concentrations corresponding to 1-5 times the LoB value. The samples were measured over 3 days in 5 replicates each day to produce 60 values. The LoD was calculated using parametric analysis. The study was performed with 1 reagent lot.

### Limit of Quantitation (LoQ)

**cobas c** 303 analyzer: 30 µg/g

**cobas c** 503 analyzer: 30 µg/g

The LoQ was established according to the CLSI guideline EP17-A2 with 4 low level stool specimen extracts. The samples were measured over 3 days in 5 replicates each day to produce a total of 15 replicates per sample. The study was performed with 1 reagent lot. The LoQ was defined as the lowest calprotectin concentration, which can be determined with a precision of < 20% CV using an acceptance criterion of LoQ ≤ 30 µg/g.

### Linearity

The linear range of the fCAL turbo was determined according to the CLSI guideline EP06-A. To demonstrate linearity, 1 dilution series, with 11 different calprotectin levels, covering and exceeding the expected measuring range of the test, was generated by blending a low and high specimen extract pool. Nine intermediate concentration levels spanning the anticipated linear range of the assay were produced. Each sample was tested in 4 replicates, using 1 reagent lot. The linear range was defined as the concentration interval in which coefficients of the non-linear, polynomial fits were determined as not significant or as the concentration interval in which the deviation of the polynomial fit from linearity was < 10 %. For values < 75 µg/g an absolute difference of < 7.5 µg/g was allowed.

| Measuring range tested [µg/g] | Linear regression parameters | | | Linear range [µg/g] |
|---|---|---|---|---|
| | Intercept | Slope | $R^2$ | |
| 17.7-13427 | 23.11 | 13429 | 1.000 | 17.7-13427 |

Table 10: Linearity study results on the **cobas c** 303 analyzer

| Measuring range tested [µg/g] | Linear regression parameters | | | Linear range [µg/g] |
|---|---|---|---|---|
| | Intercept | Slope | $R^2$ | |
| 18.7-12988 | -17.05 | 12977 | 1.000 | 18.7-12988 |

Table 11: Linearity study results on the **cobas c** 503 analyzer

The following performance characteristics have been established on the master system **cobas c** 501 analyzer. The results are also considered applicable to the **cobas c** 303 analyzers and **cobas c** 503 analyzers.

### High-dose hook effect

No high-dose hook effect was observed for samples with theoretical concentrations up to 45715 µg/g. The presence of a high-dose hook effect was tested on 1 lot.

### Interfering substances

The susceptibility of the fCAL turbo assay to oral pharmaceuticals, nutritional supplements, hemoglobin as well as enteropathological microorganisms was assessed according to the CLSI guideline EP07-A2. Bias in results exceeding 10 % was considered as interference. No interference was detected with substances listed in Table 12. No interference was detected with enteropathological microorganisms listed in Table 13, up to the indicated amounts of colony forming units (CFU) per mL of stool specimen extract.

| Trade name | Active component | Concentration in 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 |
| Sulfameth-oxazole | Sulfamethoxazole | 1.60 |
| Trimethoprim | Trimethoprim lactate | 0.35 |
| Ciproxine | Ciprofloxacin | 1.25 |
| Vitamin E | DL-α-Tocopherol acetate | 0.30 |
| Bion 3 | 3 probiotics ($10^7$ 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 12: Interfering substances: oral pharmaceuticals, nutritional supplements, hemoglobin

| Name | Final Concentration (CFU/mL) |
|---|---|
| *Escherichia coli* | $3.3 \times 10^7$ |
| *Salmonella enterica subsp. enterica* | $9.0 \times 10^7$ |
| *Klebsiella pneumoniae subsp. pneumoniae* | $5.3 \times 10^7$ |
| *Citrobacter freundii* | $12.9 \times 10^7$ |
| *Shigella flexneri* | $5.0 \times 10^7$ |
| *Yersinia enterocolitica subsp. enterocolitica* | $9.8 \times 10^7$ |

Table 13: Interfering substances: enteropathological microorganisms

### Limitations

- Test results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.
- False negative results could occur in patients who have granulocytopenia due to bone marrow depression.
- Some patients taking non-steroidal anti-inflammatory drugs (NSAID) will have elevations in their fecal calprotectin levels.
- Results may not be clinically applicable to children less than 4 years of age who have mildly increased fecal calprotectin levels.
- Patients with IBD fluctuate between active (inflammatory) and inactive stages of the disease. These stages must be considered when interpreting results of the fecal calprotectin assay.

**Special wash programming required by the instrument manufacturer:** The manufacturer of the **cobas c** systems considers the use of special wash steps as mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet provided by the instrument manufacturer. For further instructions, refer to the operator's manual.

### References

1 Occhipinti K, Smith JW. Clin Colon Rectal Surg 2012;25(1):46-52
2 Pavlidis P, Chedgy FJ, Tibble JA Scand J Gastroenterol. 2013;48(9):1048-54.
3 Mindemark M, Larsson A. Clin Biochem. 2012;45(7-8):552-5
4 Waugh N, Cummins E, Royle P et al. Health Technol Assess. 2013;17(55):xv-xix, 1-211
5 Kappelman MD, Moore KR, Allen JK et al. Dig Dis Sci. 2013;58(2):519-25
6 Stange EF, Travis SP, Vermeire S et al. J Crohns Colitis 2008;2(1):1-23
7 Van Assche G, Dignass A, Panes J et al. J Crohns Colitis 2010;4(1):7-27
8 Tibble JA, Sigthorsson G, Foster R et al. Gastroenterol 2002;123(2):450-460.
9 Jahnsen J, Røseth AG, Aadland E. Tidsskr Nor Laegeforen 2008;128:743-5
10 Dale I, Brandtzaeg P, Fagerhol MK et al. Am J Clin Pathol 1985;84(1):24-34
11 Røseth AG, Aadland E, Jahnsen J et al. Digestion 1997;58(2):176-180
12 Røseth AG, Fagerhol MK, Aadland E et al. Scand J Gastroenterol 1992;27(9):793-798
13 Fagerhol MK. Lancet 2000;356(9244):1783-4
14 Konikoff MR, Denson LA. Inflamm Bowel Dis. 2006;12(6):524-34.
15 Van Rheenen PF, Van de Vijver E, Fidler V. BMJ 2010;341:c3369. doi: 10.1136/bmj.c3369.
16 Burri E, Beglinger C. Swiss Med Wkly 2012;142:w13557. doi: 10.4414/smw.2012.13557. eCollection 2012.

### Shipping damage

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

### Incident reporting

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

### Symbols

The following symbols and signs in addition to those listed in the ISO 15223-1 standard are used (for USA: see dialog.roche.com for definition of symbols used):

| | |
|---|---|
| CONTENT | Contents of kit |
| → | Volume for reconstitution |
| GTIN | Global Trade Item Number |

# FCAL

**fCAL turbo**

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