Calprotectin CLIA Kit

Instructions for Use (IFU)

For In Vitro Diagnostic Use in Canada.

REF **80-CALHU-FC100**

Manufactured By:
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Intended use

The ALPCO Calprotectin CLIA kit is an *in vitro* chemiluminescent immunoassay (CLIA) for the quantitative measurement of fecal calprotectin in human stool. This kit is intended to be used with the KleeYa Instrument. For *in vitro* diagnostic use in Canada as an aid in the diagnosis of inflammatory bowel disease and the differentiation of inflammatory bowel disease from irritable bowel syndrome in conjunction with other clinical and laboratory findings.

Principles of the Procedure

The ALPCO Calprotectin CLIA is a chemiluminescent immunoassay system with detection based on the use of monoclonal antibodies against calprotectin. The KleeYa system allows for complete automation of immunoassays. It performs the sample processing (sample pre-dilutions, sample and reagent dispensing, incubations, wash processes) as well as the measurement and evaluation. A mouse IgG monoclonal capture antibody (mAb), highly specific to the calprotectin heterodimeric and polymeric complexes, is coated onto the magnetic beads. Calprotectin present in the sample extract is bound by the antibody conjugated to the surface of the magnetic bead. Re-calibrators, controls and sample extracts are incubated. After a washing step, a chemical-conjugated secondary mouse IgG monoclonal detection antibody detects the calprotectin molecules bound to the antibody-coated magnetic beads. After incubation and an additional wash step, a chemiluminescent substrate is added. Upon reaction, a flash of signal is directly read on the KleeYa instrument. The intensity of the light is proportional to the amount of conjugate bound, and thus to the amount of captured calprotectin. Concentration of calprotectin in the samples is calculated on the instrument based on the calibration curve as determined by the re-calibrators run within a 24-hour period.

Kit Concept

The ALPCO Calprotectin CLIA is specifically designed for automated analysis using the KleeYa instrument. To perform analysis on the instrument, carefully follow maintenance and operating instructions in the KleeYa user manual.

Reagents Supplied for 100 Tests

1 x REAGENT CARTRIDGE	**Magnetic Beads** 1 vial of magnetic beads conjugated with mouse monoclonal antibody against calprotectin. Ready to use.	1 x 5ml
	Conjugate 2 vials with the diluted enzyme-conjugated mouse monoclonal detector antibody against calprotectin. Ready to use.	2 x 10ml
	Assay Buffer 2 vials of calprotectin assay buffer. Ready to use.	2 x 10ml
2 x ANCILLARY CARTRIDGE	**Assay Buffer** 2 x 4 vials of calprotectin assay buffer. Ready to use.	8 x 10ml

ANCILLARY MATERIALS

- *USB Storage Device (10-CLUSB-01)

  *Each kit lot is supplied with a Universal Serial Bus (USB) Storage Device containing the lot-specific Definition file (a parameter file defining all the automated steps of the assay). The data from the USB Storage Device is uploaded onto the KleeYa via the USB port prior to assay run. Please refer to the instructions enclosed with the USB Storage Device (10-CLUSB-01) for uploading of lot-specific assay files.

Materials and Equipment Required but Not Provided

ALPCO Product #	Item Name	Pack Size
80-CALHU-FCSET	**1 set of Re-Calibrators** 2 vials, each containing calprotectin at a fixed concentration. Ready-to-use.	2 x 1ml
	1 set of Controls 2 vials, each containing calprotectin at a fixed concentration. Ready-to-use.	2 x 1ml
15-10111184	KleeYa Trigger Pack: 3 sets of trigger solutions	3,000 tests
15-10111002	Wash Buffer 5X	4 x 2L
15-10033666	Stackable Cuvettes; 1,000 µL	3,000 cuvettes
15-10036506	Disposable Anchor Tips; 300 µL	5,760 tips
	Distilled or Deionized Water	
	KleeYa Platform	
	General laboratory equipment	

Additional Materials Required but Not Provided

Feces sample collection

1. Sample collection tube
2. Transport container

Manual feces preparation

1. Disposable, breakable sterile inoculation loops or wooden stick
2. Disposable polypropylene screw cap tubes, 14 ml
3. Eppendorf tubes (1 - 1.5 ml)
4. Sensitive digital scale (40-150 mg) – if manual weighing
5. Extraction buffer (10-EXBUF-225)
6. Vortex mixer or shaker
7. Centrifuge (1000 – 3000 x *g*)
8. Freezer (-20ºC, -80ºC)

Device Extraction Method:

1. Easy Stool Extraction Device (30-EZEX-100)
2. Eppendorf tubes (1 - 1.5 ml)
3. Vortex mixer or shaker

4. Centrifuge (1000 – 3000 x *g*)
5. Freezer (-20ºC, -80ºC)

Precautions and Warnings

1. For *in vitro* diagnostic use in Canada.
2. Follow universal precautions. Materials of human origin used in this kit have been tested and confirmed negative for HBsAg and anti-HIV I and II and anti-HCV antibodies. They should be treated as a potential biohazard and handled and disposed of according to local laboratory legislation.
3. Warning: do not interchange components from the different kit lots. Satisfactory performance of the test is guaranteed only when components from the same batch of the Calprotectin Flash Chemiluminescence Assay are used.
4. Routine maintenance of the KleeYa instrument is required for proper performance.
5. All reagents contain ProClin 300 as a preservative agent (< 0.10%).
6. The ALPCO extraction device should not be used for the extraction of liquid/watery stool samples. Samples of this consistency should be extracted using the manual weighing extraction method.
7. Stool samples are generally heterogeneous. Mechanical homogenization using an applicator, inoculation loop, or similar device prior to sampling is recommended.
8. EASY Stool Extraction Devices may only be loaded into KleeYa racks containing the small black spacers. Re-calibrators and controls may only be loaded into a KleeYa sample rack containing white spacers. Contact ALPCO for information on loading other tube types.
9. For information on hazardous substances included in the kit and actions to take in the event of direct contact or ingestion, refer to the Safety Data Sheet available at www.alpco.com or upon request.
10. Dispose of unused reagents according to local and federal regulations.

Storage and Transport Conditions

1. Reagents are shipped on cold packs but do not require specific temperature control. Reagents should be stored at 2-8°C. Unopened reagents are stable until the expiration date on the box label. **Do Not Freeze.**
2. The expiration date is printed on all component labels.
3. When cartridges are not in use, it is recommended they are stored at 2-8ºC.

Reagent Stability

Reagent and Ancillary Cartridges
Store at 2-8ºC. Unopened cartridges are stable until the expiration date printed on the label. Opened cartridges are stable for up to four months if stored at 2-8°C if within the expiration date printed on the label.

Re-calibrators and Controls
Store at 2-8ºC. Un-opened re-calibrators and controls are stable until the expiration date printed on the label. Re-calibrators and controls must be returned to 2-8ºC when not in use. When stored properly, opened re-calibrators and controls are stable for up to 7 uses within 30 days.

Note: Ensure caps are placed on the correct vials after each use. Switching caps for re-calibrators and controls can negatively impact results.

Sample Collection

Loose or liquid stool samples are acceptable as normalization to stool weight is part of the calculation of the result. Submission of stool samples from diapers should be avoided unless the sample submitted can be taken from a portion of the stool which is not in contact with the diaper material.

Sample requirements:

1. Collection of a minimum of 1g of stool in a clean screw-top vial is recommended. No preservative is necessary or indicated.
2. Sample transport: Stool specimen should be received by the laboratory within 4 days of collection. Temperature during shipment should not exceed 28ºC. Samples must be stored at 2-8ºC upon receipt and extracted or frozen within 7 days.
3. Sample storage: Samples may be stored at 2-8ºC for up to 7 days before extraction and testing. If samples will not be tested within 7 days, freeze samples at -20ºC or -80ºC. Freezing at -80ºC is recommended for long-term storage. Stool samples may be subject to no more than 3 freeze/thaw cycles.

Reagent Preparation

1. Reagent cartridges are ready to use. The KleeYa instrument will read and upload reagent cartridge information. Bead solutions are continuously mixed onboard and the instrument will maintain reagents at 8-10°C.
2. Controls and re-calibrators are ready-to-use. Mix by gentle inversion and load in appropriate KleeYa sample racks. Make sure no bubbles are present after mixing. If necessary, assign the lot specific assay to the appropriate control(s) on the KleeYa instrument. Deselect the default sample dilution in the dilution tab for controls 1 and 2.

Sample Preparation: Stool Extraction and Dilution

Stool samples can be extracted by using:

A. The Manual Weighing / Standard Extraction Procedure or
B. The ALPCO Extraction Device Procedure

A) The Manual Weighing / Standard Extraction Procedure:

The Manual Weighing/Standard Extraction Procedure is described below in **Steps A1 to A6.**

A1. Label and weigh (tare) the empty polypropylene tube together with the inoculation loop.
A2. Take out 50 to 100 mg of the stool sample by means of the inoculation loop and place it into the pre-weighed tube.
A3. Estimate the net amount of sample, break off the inoculation loop and leave the lower part of the loop in the tube. Add Extraction Buffer (99 times the weight volume) to the tube and close the tube.
A4. Homogenize the sample on a multi-tube vortex by vigorous shaking (at highest speed) for 30 minutes.
A5. Centrifuge the extract in the tube for 5 minutes at 3000 x g.
A6. Decant the supernatant into a fresh labeled tube and continue with the assay procedure within 48 hours, or store the extracts at 2-8°C or at ≤ -80°C for up to 17 days. This extraction procedure results in a 1:100 dilution.

B) The ALPCO Extraction Device Procedure:

Please refer to the detailed instructions provided with the Easy Stool Extraction Device (30-EZEX-100). Refer to steps A4 through A6 above for final preparation of stool extracts. The extraction device procedure results in a 1:100 stool sample dilution.

Note: The manual weighing method must be used for liquid stool samples.

Following centrifugation, EASY Stool Extraction Devices can be loaded directly into KleeYa sample racks containing the small black spacers. Sample racks containing the larger white spacers may only be used for re-calibrators and controls. Extraction devices must contain a volume between 600 µL and 2,000 µL for testing on the KleeYa.

Assay Procedure

1. Tap on the Load button in the main menu bar to show the Load screen.
2. Tap on the Samples button below the Load button.
3. Load all re-calibrator, control, and sample tubes into sample rack(s).
4. Load the sample rack(s), one after the other, into the sample loading bay
5. If necessary, assign the lot specific assay to the appropriate control(s). Deselect the default sample dilution in the dilution tab for controls 1 and 2. If necessary, assign desired tests to the samples.
6. Tap on the Reagents button below the Load button.
7. Load all necessary Reagent Cartridges into reagent rack(s).
8. Load the reagent rack(s), one after the other, into the reagent loading bay
9. Load cuvettes, tips, and solutions and empty waste as required to complete the run.
10. Tap on the Start button to start the test run.

    NOTE: Any additional information can be found in the KleeYa user Manual.

Assay Calibration and Quality Control

1. Re-calibrators must be run every 24 hours of calprotectin testing in order to generate a new calibration curve.

2. Controls 1 and 2 are to be run each day calprotectin testing is performed. Calprotectin control results reported by the KleeYa software must be multiplied by 50 prior to comparison to the control range reported in the enclosed Certificate of Analysis.

3. Traceability: No international reference material or reference measurement procedures are available for calprotectin. The Calibrators and Controls used in the ALPCO Calprotectin CLIA are traceable to internal reference standards made from native calprotectin antigen diluted in a buffer containing stabilizers and preservatives.

Calculation of Results

The ALPCO Calprotectin CLIA results are directly analyzed in the KleeYa software. The concentration of calprotectin in the samples is calculated on the instrument via the calibration curve as determined by the re-calibrators within a 24-hour period. Results can be exported locally to a USB.

Performance Characteristics

Limit of Blank (LoB): The LoB was determined to be 2.8 µg/g by the nonparametric method.

Limit of Detection (LoD): The LoD was determined to be 6.5 µg/g by the parametric method.

Limit of Quantitation (LoQ): The LoQ was determined to be 8.4 µg/g.

Within-Run Precision: Mean of 6.4% CV and range of 3.8 to 11.7% CV for 8 samples.

Lot to Lot Reproducibility: Mean of 6.2% CV and range of 0 to 11.9% CV for 7 samples.

Site-to-Site Reproducibility: Mean of 4.2% CV and range of 0 to 11.1% CV for 7 samples.

Recovery:

Mean of 99.9% and a range of 89.3% to 106.3% for 7 samples spanning the AMR of the assay.

Linearity:

The ALPCO Calprotectin CLIA was found to be linear across the analytical measuring range of 8.4 µg/g to 10,000 µg/g.

Ancillary Devices

Both the pre-filled Easy Stool Extraction Device (Product# 30-EZEX-100) and the Extraction Buffer, 4X (Product# 10-EXBUF-225) for manual stool processing have been validated for use

with the ALPCO Calprotectin CLIA kit. Please contact ALPCO regarding the compatibility of additional extraction devices and buffers.

Symbols Glossary

	Product reference or catalog number
	Batch or lot number
	Temperature limitation: store at 2 to 8°C
	Expiration or use by date
	Consult instructions for use

References:

1. Manz et al. (2012). Value of Fecal Calprotectin in the Evaluation of Patients with Abdominal Discomfort: An Observational Study. BMC Gastroenterol. PMID: 22233279.

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