EPITOPE DIAGNOSTICS, INC.

EDI™ Human Calprotectin CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the quantitative measurement of Human Calprotectin in Feces.

REF CL0849 CE IVD 50, 100, 250 +2 +8 °C

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of human calprotectin levels in feces using the ECL100 or ECL25 Immunoassay analyzer. This test is used in evaluating patients suspected of having a gastrointestinal inflammatory process; distinguishing inflammatory bowel disease from irritable bowel syndrome, when used in conjunction with other diagnostic modalities, including endoscopy, histology, and imaging. It also assesses the effectiveness of IBD treatment and recurrence.

For in-vitro diagnostics purpose only

SUMMARY OF PHYSIOLOGY

Calprotectin consists of mammalian proteins S100A8 and S100A9 and is a 24 kDa dimer[1]. It is secreted during the inflammatory response in the intestinal lumen through leukocyte shedding, active secretion, cell disturbance, and cell death[7]. Thus, elevated fecal calprotectin levels are correlated with migration of neutrophils into the intestinal mucosa[3,4]. Clinical applications may include aiding in the diagnosis of ulcerative colitis[2], inflammatory bowel diseases (IBD)[6], irritable bowel syndrome (IBS)[8], and Crohn's disease[5].

ASSAY PRINCIPLE

This CLIA is designed, developed, and produced for the quantitative measurement of human calprotectin in fecal samples. The assay utilizes a two-site "sandwich" technique with two antibodies that bind to different epitopes of calprotectin.

Assay calibrators, controls, or patient samples are added directly to a reaction vessel containing streptavidin coated magnetic particles. Simultaneously, an acridinium ester antibody and a biotin antibody are added. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of "magnetic particles – biotin calprotectin antibody –calprotectin – acridinium ester calprotectin antibody".

The materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the trigger solution is added to the reaction vessel and light generated by the reaction is measured with the ECL100 or ECL25 analyzer. The relative light units (RLU) are proportional to the concentration of calprotectin in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in fecal calprotectin concentration.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.Reagents from different kit lot numbers should not be combined or interchanged.

Standard Batch Quantity: 100/kit

1. Calprotectin Magnetic Particle Solution (L0501)

Qty: 1 x 2.0 mL (50/kit), 1 x 2.3 mL (100/kit), 1 x 5.4 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

2. Biotin Calprotectin Antibody (L0502)

Qty: 1 x 3.5 mL (50/kit), 1 x 6 mL (100/kit), 1 x14 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

3. Acridinium Ester Calprotectin Antibody (L0503)

Qty: 1 x 6.0 mL (50/kit), 1 x 11 mL (100/kit), 1 x 26.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

4. Calprotectin Calibrators(L0504 – L0505)

Lyophilized human calprotectin a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty: 2 x vials
Storage: 2 – 8°C before reconstitution, <-20°C after reconstitution
Preparation: Must be reconstituted with 1.0 mL of demineralized water and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely and there are no air bubbles prior to use.

5. Calprotectin Controls (L0506 – L0507)

Lyophilized human calprotectin in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty: 2 x vials
Storage: 2 – 8°C before reconstitution, <-20°C after reconstitution
Preparation: Must be reconstituted with 1.0 mL of demineralized water and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely and there are no air bubbles prior to use.

6. Concentrated Fecal Extraction Buffer (31301) (Optional)
Not provided in the kit: If needed, please order separately.

Concentrated buffer matrix with protein stabilizers and preservative which serves as a patient sample diluent containing a surfactant in phosphate-buffered saline with a non-azide preservative.

Qty: 1 x 120 mL
Storage: 2 – 8°C

Preparation: 5X Concentrate. The contents must be diluted with 480 mL distilled water and mixed well before use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

The instrument only uses materials supplied by Epitope Diagnostics, Inc. When materials available from third-party suppliers are used, Epitope Diagnostics, Inc. takes no responsibility for the validity of results obtained. Material is available for purchase from Epitope Diagnostics, Inc. Please contact your distributor for more information.

1. ECL100 Immunoassay Analyzer or ECL25 Immunoassay Analyzer
2. CL011 Cuvettes (for ECL100) or CL010 Cuvettes (for ECL25)
3. Wash Reagent (P-594)
4. Trigger Solutions A and B (P-595)
5. EDI™ q-FOB™, PE-1 and CP Fecal Sample Collection Kit (30210)

SPECIMEN COLLECTION AND PREPARATION

Fresh fecal sample should be collected in a stool sample collection container by patients. It is advised to collect a minimum of 1-2 mL liquid stool specimens or 1-5 g solid stool specimens.

Specimen transport: After collection, stool specimens should be stored at 2–8 °C prior to shipment and must be received by the laboratory for processing within 3 days of collection. Stool specimens may be shipped at room temperature or on cold packs.

Specimen storage: Stool specimens should be refrigerated at 2-8 °C and extracted within 3 days of receipt at the laboratory.

Specimen extraction: Fecal sample should be further collected and extracted in EDI™ q-FOB™, PE-1 and CP Fecal Sample Collection Kit (30210) in clinical laboratory. The tube is specifically designed for the easy collection/extraction of a substantial and consistent amount of a fecal sample into sample extraction buffer pre-filled tube:

1. Open the lid of primary stool collection container
2. Open the device sampling lid (purple color) and slowly pull the sampling lid out of the tube.
3. Use the grooved region of the wand to stir and mix soft stool or dip into hard stool multiple times (3 - 5 sites) to collect a small amount of stool sample. The whole grooved region of the wand must be covered with stool sample.
4. Insert the sampling wand and screw the sampling lid back into the sampling tube in a vertical position. Tightly seal the sampling lid against the tube. Let the sample soak in extraction buffer at least 10 minutes.
5. Vortex the tube on upright position at least 10 seconds to dissolve the stool sample completely. It is important to ensure that the grooved region of the wand is completely free of stool sample residue. (A digital roller mixer can be used to assist in facilitating the mixing process.)
6. Place the extracted sample upright on a rack for 1 to 5 minutes to allow stool particles to settle. If bubbles or foam are present, remove them before performing the assay.

Extract storage: The extracted stool specimens should be tested within 8 hours or stored at 2-8 °C if testing will occur within 3 days. It may be stored below - 20 °C for a longer storage period. Avoid more than three freeze - thaw cycles for each specimen.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration must be performed when a reagent lot is used for the first time and remains valid for 28 days. After this period, recalibration is required. Additionally, we recommend performing calibration if control results fall outside the acceptable range.

QUALITY CONTROL

The use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. It is strongly recommended to perform a control test before running patient samples. If no patient samples are tested, a control test is not necessary. Quality control results that fall outside the acceptable range may indicate invalid test results. Please refer to the Certificate of Analysis for the correct control range.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator and control vials.

2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges.

2.2 For the ECL100, take out the Magnetic Particle bottle make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance.

- Note: For ECL100, if the Magnetic Particle Solution volume is over 3 mL, it will be provided in a glass bottle. It will need to be transferred from the glass bottle to the plastic vial in the cartridge with the rest of the reagents. Make sure the Magnetic Particle Solution is mixed well before transferring.

2.3 For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber.

2.4 The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
Calprotectin Calibrators (L0504-L0507)	75	-
Samples	-	75
Biotin Calprotectin Antibody (L0502)	50	50
Calprotectin Magnetic Particle Solution (L0501)	20	20

Incubation Period 1		
Wash the reaction cup 3 times with the wash reagent.		
Acridinium Ester Calprotectin Antibody (L0503)	100	100
Incubation Period 2		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	200	200
Trigger Solution B (P-595)	200	200

The assay total incubation time is less than 30 minutes.

INTERPRETATION OF RESULTS

The chemiluminescence analyzer calculates the concentration values of the sample and the control by a standard curve (fitting method: four parameters or point-to-point) and the measured RLU. Values are compared with the range of the marked value. If it exceeds the indicated quality control range, it indicates that the test is unqualified and needs to be re-tested.

Due to methodological differences or antibody specificity, there may be deviations between the test results of reagents from different manufacturers. Therefore, direct comparisons should not be made to avoid false interpretation.

EXPECTED VALUES

Calprotectin concentrations were measured in stool samples collected from 125 apparently healthy adults using the EDI™ Fecal Human Calprotectin CLIA Kit. The observed range of calprotectin is summarized in the table below.

	Calprotectin Concentration
Normal	0 – 50 µg/g
Light Positive	50 – 100 µg/g
Positive	100 – 200 µg/g
Strong Positive	>200 µg/g

It is highly recommended that each laboratory should establish their own normal range for calprotectin concentration based on local populations.

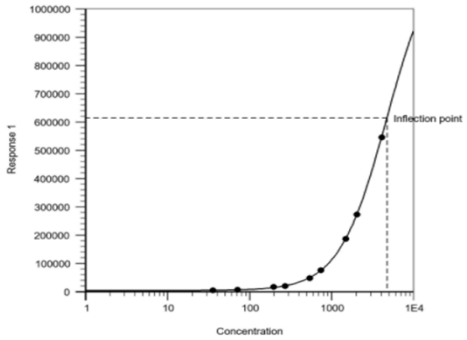
LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL25 Immunoanalyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different lots cannot be mixed.
3. Test results from this product should not be the sole basis for clinical diagnosis.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measurement result is recalculated according to the dilution ratio to ensure the accuracy of the result.

PERFORMANCE CHARACTERISTICS

Example of Calibration Curve

The calibration curve is built in the calibration card. This curve is lot dependent. Here is an example of the 10-point calibration curve.



Hook Effect

The assay shows no hook effect up to 93,567.2 µg/g.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates in three assays of calibrator matrix to be 0.20 µg/g.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates in three assays of low-level samples to be 2.3 µg/g.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates in three assays of low-level samples to be 4.4 µg/g.

Linearity

The linearity of an assay was determined in a duplicate by the serial dilutions of a test specimen. The test correlation (R^2 = 0.99) was observed by analyzing measured Calprotectin concentration against the theoretically calculated Calprotectin concentration using a linear regression. The linearity of this test is up to 5000 µg/g. The results are summarized below:

Standard	Average Concentration (µg/g)	Theoretical Concentration (µg/g)	Linear Recovery (%)
Matrix	0	0	-
1.6%	74.5	82.0	91%
3.1%	163.8	164.0	100%
6.3%	359.4	328.0	110%
12.5%	736.1	656.0	112%
25%	1537.0	1495.0	103%
50%	2854.5	2990.0	95%
100%	5427.0	5980.0	91%

Intra-assay Repeatability

Repeatability was determined by measuring eight replicates of three extracted stool specimens. The results are as follows:

Sample	Average Concentration(µg/g)	SD	CV (%)
1	29.0	0.98	3.4%
2	400.1	5.63	1.4%
3	1115.9	28.95	2.6%

Inter-assay Reproducibility

Reproducibility was determined by measuring three specimens in twenty-four replicates over the run of three assays. The results are summarized below:

Sample	Average Concentration (µg/g)	SD	CV (%)
1	28.9	0.81	2.8%
2	405.4	8.56	2.1%
3	1166.46	32.82	2.8%

Cross Reactivity

Cross-reactivity was assessed by analyzing several specimens containing similar analytes at elevated concentrations. The results are summarized below:

Analytes	Theoretical Concentration	Measure Concentration (µg/g)
MPO Antigen	994 µg/g	< 0.010
Osteocalcin Antigen	68.2 ng/mL	< 0.010
Hemoglobin-Haptoglobin Complex	649.3 µg/g	< 0.010
Chromogranin A Antigen	7977.3 ng/mL	< 0.010

Interference

Bilirubin, hemoglobin and lipid triglycerides were tested as potential interferents to Fecal Calprotectin. Randomly selected samples were spiked with the potential interferents at the concentrations listed in the table below:

Interferent (Concentration tested, mg/mL)		Test (µg/g)	Control (µg/g)	Bias (d_{obs}, %)
Bilirubin	0.005	268.80	277.30	-2.4%
	0.01	292.05	277.30	5.3%
	0.02	271.25	277.30	-2.2%
Hemoglobin	0.5	273.20	274.05	-0.3%
	1.0	277.10	274.05	1.1%
	2.0	287.35	274.05	4.9%
Lipids	1.0	263.10	272.30	-3.4%
	5.0	271.25	272.30	-0.4%
	10.0	276.50	272.30	1.5%

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD — In Vitro Diagnostic Device

CE — European Conformity

LOT — Lot Number

REF — Catalog Number

Read instructions before use

Number of Tests

Store at

Use by

Keep away from heat and direct sun light

Manufacturer

EC REP — Authorized Representative in Europe