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

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 (30473) (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. Calprotectin Sample Collection Tube kit (KT-843)

SPECIMEN COLLECTION AND PREPARATION

Fresh fecal sample should be collected in a stool sample collection container by patients. It is advised to collect minimum of 1-2 mL liquid stool sample or 1-5 g solid stool sample. The sample should be transported to the lab in a frozen condition (-20°C). The sample is allowed to be stored at 2-8°C if it is intended to be tested on a day of sample collection.

Fecal sample should be further collected and extracted in EDI™ Calprotectin Sample Collection Tube Kit (KT-843) 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. After collection, allow the tube to be sitting upright position for 3 - 10 minutes and then vortex the tube to dissolve all the feces. There should not have any feces stuck to the collection wand. Should the extracted sample be tested immediately, **please make sure that all the foam/bubble must be removed and the solid feces should be sedimented to the bottom of the tube**. A brief centrifugation procedure may be helpful to remove the bubble and to sediment fecal particles. The extracted fecal sample should be loaded on ECL100 and should be tested within 24 hours. The extracted fecal sample may be stored below -20 °C for retention purpose. Avoid more than three freeze-thaw cycles for each specimen.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration is required for the first-time use of a reagent lot and is valid for 28 days. However, we recommend calibration every 14 days after initial calibration or when either kit control is out of range.

QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random access format. Use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. We suggest performing a control test once every day. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

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 ECL 100, 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

Hook Effect

The assay shows no hook effect up to 100,000 µg/g.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates in three assays of calibrator matrix to be 0.70 µg/g.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates in three assays of low-level samples to be 1.60 µg/g.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates in three assays of low-level samples to be 2.51 µg/g.

Linearity

Linearity was determined by two assays with a diluted standard of high calprotectin concentration. In each assay, the average of two replicates of each of the diluted samples is used for a correlation analysis against calculated theoretical values. The linearity of this test is up to 5000 µg/g.

Standard	Average Concentration (µg/g)	Theoretical Concentration (µg/g)	Linear Recovery (%)	R
1	0	0	-	0.9992
2	74.5	82.0	91%	
3	183.8	164.0	100%	
4	359.4	328.0	110%	
5	736.1	656.0	112%	
6	1537.0	1495.0	103%	
7	2854.5	2990.0	95%	
8	5427.0	5980.0	91%	

Repeatability

Reproducibility was determined by measuring ten replicates of controls.

Control	Average Concentration (µg/g)	CV (%)
1	196.8	2.4
2	50.0	4.1

Accuracy

Accuracy was determined by three replicates of two middle standards used to generate the multi-point calibration curve.

Standard	Measured Concentration (µg/g)	Average Concentration (µg/g)	Target Value ± 15% (µg/g)
4	64.7	64.5	54.9 – 74.2
	69.0		
	68.0		
5	225.0	225.5	191.7 - 258.8
	219.2		
	225.6		

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.

REFERENCES

1. Brophy, Megan Brunjes; Nolan, Elizabeth M. (2015). "Manganese and Microbial Pathogenesis: Sequestration by the Mammalian Immune System and Utilization by Microorganisms". *ACS Chemical Biology*. **10**: 150116125412006. doi:10.1021/cb500792b. PMC 4372095.
2. Costa, F.; Mumolo, M. G.; Ceccarelli, L.; Bellini, M.; Romano, M. R.; Sterpi, C.; Bottai, M. (2005). "Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease". *Gut*. *54* (3): 364–368. doi:10.1136/gut.2004.043406.
3. Gupta, Ramesh (2014). Biomarkers in toxicology. San Diego, CA: Academic Press. pp. 272–273. ISBN 9780124046498.
4. Striz, I.; Trebichavský, I. (2004-01-01). "Calprotectin - a pleiotropic molecule in acute and chronic inflammation". Physiological Research / Academia ScientiarumBohemoslovaca. 53 (3): 245–253. ISSN 0862-8408. PMID 15209531.
5. Tibble, J.; Teahon, K.; Thjodleifsson, B.; Roseth, A.; Sigthorsson, G.; Bridger, S.; Bjarnason, I. (2000). "A simple method for assessing intestinal inflammation in Crohn's disease". *Gut*. *47* (4): 506–513. doi:10.1136/gut.47.4.506.
6. Vaos, G.; Kostakis, ID.; Zavras, N.; Chatzemichael, A. (2013). "The role of calprotectin in pediatric disease". Biomed Res Int (Review). 2013: 542363. doi:10.1155/2013/542363. PMC 3794633. PMID 24175291.
7. Walsham, Natalie E.; Sherwood, Roy A. (2016). "Fecal calprotectin in inflammatory bowel disease". Clinical and Experimental Gastroenterology. 9: 21–29. doi:10.2147/CEG.S51902. ISSN 1178-7023. PMC 4734737. PMID 26869808.
8. Waugh, N.; Cummins, E.; Royle, P.; Kandala, NB.; Shyangdan, D.; Arasaradnam, R.; Clar, C.; Johnston, R. (Nov 2013). "Faecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: systematic review and economic evaluation". Health Technol Assess (Review). 17 (55): xv–xix, 1–211. doi:10.3310/hta17550. PMC 4781415. PMID 24286461.

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.

This product is manufactured by

Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, US

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

EC	REP	MDSS GmbH Schiffgraben 41 30175 Hannover, Germany