EPITOPE DIAGNOSTICS, INC.

EDI™ Fecal Lactoferrin CLIA Kit

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

REF CL0011R RUO 50, 100, 250 +2/+8°C

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of Lactoferrin levels in feces using the ECL100 or ECL25 Immunoassay analyzer.

For Research Use only

SUMMARY OF PHYSIOLOGY

Lactoferrin is an iron-binding protein that can be secreted by non-specific inflammatory cells called neutrophils[1,2]. It is a glycoprotein consisting of approximately 700 amino acids, and can be found in mucosal secretions, plasma, tears, milk, and saliva[3]. Fecal Lactoferrin serves as a biomarker for intestinal inflammation, as the lactoferrin concentration in the gastrointestinal tract is shown to increase with the severity of inflammation[1,2]. Lactoferrin tests are particularly useful when determining if gastrointestinal symptoms are functional, such as with irritable bowel syndrome, or due to infection or inflammation, such as with inflammatory bowel disease[1]. Fecal lactoferrin tests are non-invasive ways to indicate the presence of several conditions such as ulcerative colitis, Crohn's disease, and inflammatory bowel disease[2].

ASSAY PRINCIPLE

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

Assay calibrators, controls, or patient samples are added directly to a reaction vessel containing streptavidin coated magnetic particles. Acridinium ester antibody and a biotin antibody are added. The magnetic particles capture the biotin antibody as well as an immune-complex in the form of "magnetic particles – biotin Lactoferrin antibody –Lactoferrin– acridinium ester Lactoferrin 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 Lactoferrin in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in FecalLactoferrin 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. It can be stored for 1 month at 2 – 8°C after kit opening.
Reagents from different kit lot numbers should not be combined or interchanged.

Standard Batch Quantity: 100/kit

1. Lactoferrin Magnetic Particle Solution (L0693)
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 Lactoferrin Antibody (L0694)
Qty: 1 x 3.5 mL (50/kit),1 x 6.0 mL (100/kit), 1 x14.0 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

3. Acridinium Ester Lactoferrin Antibody (L0695)
Qty: 1 x6.0 mL (50/kit), 1 x11.0 mL (100/kit), 1 x 26.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

4. Lactoferrin Calibrators (L0696 – L0697)
Liquid Lactoferrinin a BSA-based matrix with an azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials of 0.5 mL each
Storage: 2 – 8°C
Preparation: 0.5 mL of Calibrators, mix by inversions or gentle vortexing. Make sure that Calibrators are well mixed before use.

5. Lactoferrin Controls (L0698 – L0699)
Liquid Lactoferrinin a BSA-based matrix with an azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials of 0.5 mL each
Storage: 2 – 8°C
Preparation: 0.5 mL of Controls, mix by inversions or gentle vortexing. Make sure that Controls are well mixed before 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 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)

SPECIMEN COLLECTION AND PREPARATION

Fresh fecal samples 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™ 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 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 and 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 ECL25 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)
Lactoferrin Calibrators (L0696-L0697)	25	-
Samples	-	25
Biotin Lactoferrin Antibody (L0694)	50	50
Lactoferrin Magnetic Particle Solution (L0693)	20	20
Incubation Period		
Wash the reaction cuvette 3 times with wash reagent.		
Acridinium Ester Lactoferrin Antibody (L0695)	100	100
Incubation Period		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	200	200
Trigger Solution B (P-595)	200	200

The 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/antigen 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

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

	Lactoferrin Concentration
Normal	0.00 – 7.25μg/g
Elevated	> 7.25 μg/g

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

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL25Immunoanalyzer 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.
5. When the sample concentration of Lactoferrin is lower than the detection lower limit, the test result will be reported as <0.56μg/g. When the sample concentration is higher than the detection upper limit, it will be reported as >200.00μg/g.

PERFORMANCE CHARACTERISTICS

Hook Effect

The assay shows no hook effect up to 1800.00μg/g.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates in three assays of calibrator matrix to be 0.01μg/g.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates in three assays of low-level samples to be 0.56μg/g.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates in three assays of low-level samples to be 1.10μg/g.

Linearity

Linearity was determined by two assays with a diluted standard of high Lactoferrin 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 200.00μg/g.

Standard	Average Concentration (μg/g)	Theoretical Concentration (μg/g)	Linear Recovery (%)	R^2
1	0.00	0.00	-	0.998
2	2.78	3.13	89	
3	5.94	6.25	95	
4	11.19	12.50	89	
5	22.39	25.00	90	
6	51.32	50.00	103	
7	105.35	100.00	105	
8	194.60	200.00	97	

Intra-assay Precision

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

Sample	Average Concentration (μg/g)	SD	CV (%)
1	5.49	0.21	3.9
2	44.89	1.63	3.6
3	176.23	9.29	5.3

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	5.59	0.25	4.5
2	46.43	2.12	4.6
3	172.45	14.26	8.3

Cross Reactivity

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

Analytes	Theoretical Concentration	Measure Concentration (μg/g)
Calprotectin	3031.60 μg/g	0.27
Cryptosporidium Parvum	67.50 U/mL	0.43
Giardia	403.40 U/mL	0.26
PE-1	3175.20 μg/g	0.43
qFOB	1536.66μg/g	0.12
Toxin A	125.00 U/mL	0.06

Interference

Bilirubin, hemoglobin, intralipid, lysozyme, and human elastase were tested as potential interferents to Lactoferrin. Randomly selected samples were spiked with the potential interferents at the concentrations listed in the table below:

Interferent (Concentration tested)		Test (μg/g)	Control (μg/g)	Bias (d_{obs}, %)
Bilirubin	0.005 mg/mL	3.72	3.71	0.4
		61.54	62.52	-1.6
	0.01 mg/mL	3.64	3.71	-1.8
		61.34	62.52	-1.9
	0.02 mg/mL	3.70	3.71	-0.1
		67.17	62.52	7.4
Hemoglobin	0.125 mg/mL	4.00	4.04	-1.0
		73.66	70.10	5.1
	0.25 mg/mL	4.47	4.04	10.7
		74.21	70.10	5.9
	0.5 mg/mL	4.84	4.04	19.8
		72.62	70.10	3.6
Intralipid	0.25 mg/mL	4.09	4.15	-1.4
		69.33	69.08	0.4
	0.5 mg/mL	3.98	4.15	-4.1

		67.83	69.08	-1.8
	1.0 mg/mL	4.17	4.15	0.5
		61.74	69.08	-10.6
Lysozyme	10.0 ng/mL	4.58	4.64	-1.3
		66.42	64.25	3.4
	50.0 ng/mL	4.41	4.64	-5.0
		63.50	64.25	-1.2
	500.0 ng/mL	4.38	4.64	-5.5
		58.54	64.25	-8.9
Human Elastase	10.0 ng/mL	4.32	4.33	-0.2
		59.09	58.73	0.6
	100.0 ng/mL	4.17	4.33	-3.7
		67.30	58.73	14.6
	560.0 ng/mL	4.31	4.33	-0.6
		58.52	58.73	-0.3

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

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3. Liu F, Lee SA, Riordan SM, Zhang L and Zhu L (2020) Global Studies of Using Fecal Biomarkers in Predicting Relapse in Inflammatory Bowel Disease. Front. Med. 7:580803. doi: 10.3389/fmed.2020.580803

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.
17034 Camino San Bernardo
San Diego, CA 92127, 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

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)