EPITOPE DIAGNOSTICS, INC.

For Reference use only

EDI™Fecal Pancreatic Elastase-1 CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the quantitative determination of a Human Pancreatic Elastase 1in feces

REF CL0006R RUO 100, 150, 250

INTENDED USE

The EDI™ Fecal Pancreatic Elastase 1 CLIA Kit is a Chemiluminescence Immunoassay (CLIA) intended for the quantitative determination of human pancreatic elastase-1 antigen level in feces using the ECL100 or ECL25 Immunoassay analyzer.

For Research Use Only

SUMMARY OF PHYSIOLOGY

Pancreatic elastase-1 (PE-1) is a chymotrypsin-like elastase family, member 3B (CELA3B) secreted from the pancreas as a zymogen, and is responsible for digestive functions. Unlike other pancreatic enzyme, PE-1 does not degrade passing through the gastrointestinal tract and shows excellent stability. It is reported that quantification of PE-1 in stool is an indirect marker of exocrine pancreatic insufficiency (EPI), which is described as a reduction in pancreatic enzyme activity below normal digestive threshold. Patient with EPI may show a reduced PE-1 concentration in a stool.

ASSAY PRINCIPLE

The EDI™ Fecal Pancreatic Elastase 1 CLIA Kit is designed, developed, and produced for the quantitative measurement of pancreatic elastase-1antigeninfecalsamples. The assay utilizes a two-site “sandwich” technique with two antibodies that bind to different epitopes of PE-1antigen. Assay calibrators, controls, or extracted patient fecal samples are added directly to a reaction vessel. Simultaneously, a biotinylated antibody, a streptavidin coated magnetic particle andsubsequentlyacridinium ester conjugated antibodyare added to the reaction vessel. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of “magnetic particles – biotinylatedanti-PE-1 antibody–PE-1 antigen–acridinium ester conjugated anti-PE-1 antigen antibody”. Materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, trigger solutions are 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 PE-1 antigen in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in fecal PE-1 antigen concentration.

REAGENTS: PREPARATION AND STORAGE

The kit must be stored at 2 – 8°C upon receipt. All components are stable until expiration date stated over the label on kit box. Reagents from different kit lot numbers should not be combined or interchanged.

1. PE-1 Magnetic Particle Solution (L0530)

Qty: 1 x 2.8 mL (100/kit), 2 x 2.5 mL (150/kit), 3 x 2.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to use

2. Biotin PE-1 Antibody (L0531)

Qty: 1 x 11 mL (100/kit), 1 x 16.5 mL (150/kit), 1 x 26.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to use

3. Acridinium Ester PE-1 Antibody (L0532)

Qty: 1 x 11 mL (100/kit), 1 x 16.5 mL (150/kit), 1 x 26.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to use

4. Fecal PE-1 Antigen Calibrators (L0535 – L0536)

Lyophilized Fecal Pancreatic Elastase-1 in a bovine serum albumin-based matrix with an azide preservative. Refer to vials for exact concentration.

Qty: 2 x vials
Storage: 2 – 8°C before reconstitution, <-20°C after reconstitution; Do not exceed 6 freeze-thaw cycles.
Preparation: Must be reconstituted with 0.5 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. Fecal PE-1 Antigen Controls (L0537 – L0538)

Lyophilized fecal pancreatic elastase-1 in a bovine serum albumin-based matrix with an azide preservative. Refer to vials for exact concentration.

Qty: 2 x vials
Storage: 2 – 8°C before reconstitution, <-20°C after reconstitution; Do not exceed 6 freeze-thaw cycles.
Preparation: Must be reconstituted with 0.5 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 (30669) (Packaged 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 60 mL
Storage: 2 – 8°C
Preparation: 4X Concentrate. The contents must be diluted with 180 mL distilled water and mixed well before use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for research use only. 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. Exercise Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

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™ qFOB Collection Tube (30210)

The instrument must operate using materials supplied by Epitope Diagnostics, Inc. When materials are sourced from a third-party suppliers are being used, Epitope Diagnostics, Inc. takes no responsibility of the validity for obtained results. Materials are available to purchase from Epitope Diagnostics, Inc. Please contact your distributor for more information.

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 (-20ºC). The sample is allowed to be stored at 2-8ºC if it is intended to be tested on a day of sample collection.

Fecal sample should be further collected and extracted in EDI™ qFOB Collection Tube (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. 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 or ECL25 and should be tested within 6 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. Additional calibration is required every 14 days or whenevercontrols are out of a 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 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. 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. 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 for instrument operation.

Components	Quality Control Hole (µL)	Sample Hole (µL)
PE-1AntigenControls (L0537-L0538)	25µL	-
Extracted Fecal Samples	-	25µL
Biotin PE-1 Antibody (L0531)	100µL	100µL
PE-1Magnetic Particle Solution (L0530)	25µL	25µL
Incubation period 1		
Wash the reaction cup 3 times with the wash reagent		
Acridinium Ester PE-1Antibody (L0532)	100µL	100µL
Incubate period 2		
Wash the reaction cuvette 3 times with wash reagent		
Trigger Solution A (P-595A)	200µL	200µL
Trigger Solution B (P-595B)	200µL	200µL

The assay total incubation time is less than 25 minutes.

INTERPRETATION OF RESULTS

The chemiluminescence analyzer calculates the concentration values of the sample and 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 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

Fecal PE-1 antigen concentrations were measured in stool samples collected from 25 apparently healthy adults using the EDI™ Fecal Pancreatic Elastase-1CLIA Kit. The suggested normal cut off is **200µg/gstool.**EDI recommendslaboratories to establish their own normal range for fecal PE-1 antigen based on local population.

LIMITATIONS OF THE PROCEDURE

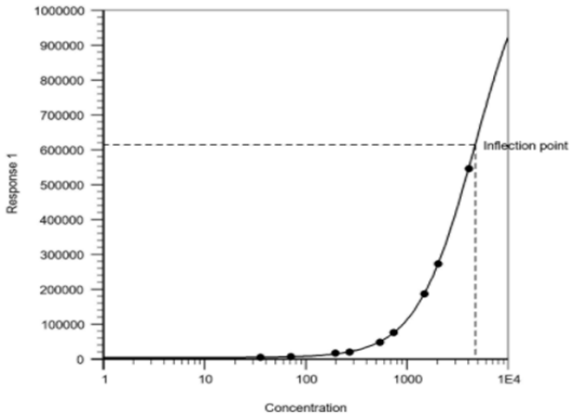
1. This product is for use on the ECL100 or ECL25 Immunoanalyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance,

instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different kit lot numbers should not be combined or interchanged
3. Test results obtained from the proposed kit should not be served as a sole basis for clinical diagnosis or patient management.
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 measured value 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 (L0534).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 1000µg/g stools.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates of standard matrix to be 1.07 µg/g.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates of low-level samples to be 1.59µg/g.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates of low-level samples to be 4.81µg/g.

Linearity

The linearity of an assay was determined in a triplicate by the serial dilutions at 80%, 60%, 40%, 20%, and 10%, of a test sample. The test correlation (R^2 = 0.99) was observed by analyzing measured PE-1 concentration against the theoretically calculated PE-1 concentration using a linear regression. The results are summarized below:

% of Dilution	Measured Concentration (µg/g)	Theoretical Concentration (µg/g)	Linear Recovery (%)
100%	404.8	538.9	106%
80%	327.2	431.1	91%
60%	247.8	323.3	95%
40%	205.4	215.6	77%
20%	98.1	107.8	76%
10%	57.3	53.9	75%

Intra-assay Precision

Precision was determined by measuring eight replicates of two extracted stool samples and one assay control.The results are as follows:

Sample	Average Concentration(µg/g)	SD	CV (%)
1	144.71	4.96	3.4%
2	151.19	5.73	3.8%
Control	394.68	13.11	3.3%

Inter-assay Reproducibility

Reproducibility was determined by measuring three control samples in sixteen replicates over the run of two assays. The results are summarized below:

Sample	Average Concentration (µg/g)	SD	CV (%)
1	147.7	3.21	2.2%
2	152.9	1.94	1.3%
3	391.9	7.36	1.9%

Repeatability

Repeatability was determined by measuring three replicates of three extracted stool samples and one assay control over the run of fourteen assays. The results are as follows:

Sample	Average Concentration (µg/g)	SD	CV (%)
1	1225.4	81.0	6.6%
2	3057.6	258.0	8.4%
3	2045.0	182.9	8.9%
Control	583.4	32.8	5.6%

Accuracy

Accuracy was determined by testing three samples in a triplicate over the run of nine tests in three assays and the obtained average concentration value should meet ± 15% of target values. The results are as follows:

Sample	Average Concentration(µg/g)	Target Value ± 15% (µg/g)	Result
1	210.47	179 – 242	Pass
2	556.90	473 – 640	Pass
3	1332.33	1132 - 1532	Pass

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. -: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. Stríz, 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 to place an order, please contact Epitope Diagnostics, Inc. at +1 (858) 693-7877 or fax to +1 (858) 693-7678 or email at cs@epitopediagnostic.com

This product is developed and manufactured by

EpitopeDiagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, USA

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

MDSS GmbH
Schiffgraben 41,
30175 Hannover, Germany

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD In Vitro Diagnostic Device	RUO For Research Use Only	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	CE European Conformity