EPITOPE DIAGNOSTICS, INC.

EDI™ Fecal Cryptosporidium parvum Antigen CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the detection of Cryptosporidium parvum in Feces.

REF CL0814 CE IVD 100, 150, 250 +2 +8 °C

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative detection of *Cryptosporidium parvum* (Crypto) antigen in feces using the ECL-100 or ECL-25. This assay is a useful tool in the aid of diagnosis of active *C. parvum* infection in acute or chronic diarrhea. This kit is for in vitro diagnostic use only.

SUMMARY OF PHYSIOLOGY

Cryptosporidiosis is one of the main causes of persistent diarrhea in the developed world. It is caused by the presence of *Cryptosporidium parvum* oocysts in the gastro-intestinal tract. This parasite is known to be highly pathogenic, and its infectious stage is transmitted by faecal-oral contact. It is also an opportunistic pathogen found in immunocompromised patients.

The symptoms of cryptosporidiosis are watery diarrhea, stomach cramps, weight loss, nausea, and fever. In industrialized countries, 2-2.5% of diarrheal hospitalized patients shed *C. parvum* oocysts. Ten percent of AIDS patients have chronic cryptosporidiosis, and this figure can be as high as 40% in developing countries. *C. parvum* is diagnosed by either Ziehl-Neelsen stain or immunofluorescence in smears of unconcentrated specimens.

ASSAY PRINCIPLE

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

Assay calibrators, controls, or patient samples are added directly to a reaction vessel containing streptavidin coated magnetic particles. An acridinium ester conjugated antibody and a biotin conjugated antibody are added. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of "magnetic particles – biotin anti-*C. parvum* antibody –*C. parvum* – acridinium ester anti-*C. parvum* 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 ECL-25 analyzer. The relative light units (RLU) are proportional to the concentration of *C. parvum* in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in Units per mL 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.

1. Crypto Magnetic Particle Solution (L0566)

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 anti-Crypto Antibody (L0567)

Qty: 1 x 6 mL (100/kit), 1 x 9 mL (150/kit), 1 x 14 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

3. Acridinium Ester anti-Crypto Antibody (L0568)

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. Crypto Calibrators (L0571-L0572)

Liquid *Cryptosporidium parvum* in a bovine serum albumin-based matrix with a sodium azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials, 1 mL each
Storage: 2 – 8°C
Preparation: Mix by inversion or gentle vortexing.

5. Crypto Controls (L0573-L0574)

Liquid *Cryptosporidium parvum* in a bovine serum albumin-based matrix with a sodium azide preservative. Refer to vials for exact concentration.
Qty: 2 x vial of 1 mL each
Storage: 2 – 8°C
Preparation: Mix by inversions or gentle vortexing.

6. Concentrated Fecal Extraction Buffer (30669) (Packed 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 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 sample should be collected into a stool sample collection container. It is required to collect a minimum of 1-2 mL liquid stool sample or 1- 5 grams solid sample. The collected fecal sample must be transported to the lab in a frozen condition (-20ºC). If the stool sample is collected and tested in the same day, it is allowed to be stored at 2-8ºC.

Patient samples need to be diluted **1:11**with patient sample diluent before being measured. A 1x working solution of concentrated fecal sample extraction buffer (30669)is suggested in the extraction of samples used with this assay.

1. Label a test tube (12x75mm) or a 2.5 mL plastic vial.
2. Add 1 mL of diluted fecal sample extraction buffer to each tube or vial.
3. Add 100 µL of liquid stool sample to the above tube.
4. With solid stool sample, take an equivalent amount (about 80-120 mg) with a spatula or a disposable inoculation loop. Vigorously mix or vortex to dissolve stool specimen in the tube.
5. Let the extracted samples sit and sediment for 15 minutes. Make sure there is not free particle on the surface of liquid supernatant. Load the tube for sample test. Alternatively, centrifuge the extracted fecal sample at 1000 rpm (200 g) for 3 minutes before loading the tube for testing.

Note: The supernatant MUST be particle free to avoid damaging the ECL100 or ECL25 instrument. If necessary, remove the supernatant into an empty tube to ensure that no particles are present.

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 every 14 days thereafter or when either kit control is out of range. Refer to appropriate system manuals for configuring calibrators.

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 and insert soft caps.

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 or ECL25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
Crypto Controls (L0573-L0574)	50	-
Samples	-	50
Biotin anti-Crypto Antibody (L0567)	50	50
Crypto Magnetic Particle Solution (L0566)	25	25
Incubation Period 1		
Wash the reaction cup 3 times with the wash solution.		
Acridinium Ester anti-Crypto Antibody (L0568)	100	100
Incubation Period 2		
Wash the reaction cup 3 times with the wash solution.		
Trigger Solution A (P-595)	200	200
Trigger Solution B (P-595)	200	200

The assay total incubation time is less than 20 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

Fecal *C. parvum* antigen concentrations were measured in stool samples collected from 55 apparently healthy adults using the EDI™ Fecal Cryptosporidium parvum Antigen CLIA Kit. The suggested positive cut off is 0.59 U/mL.

It is highly recommended that each laboratory should establish their own normal range for fecal *C. parvum* antigen based on local populations.

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL-25 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

Linearity
Linearity was determined by three replicates each of a set of 5 dilutions of the highest standard.

Standard	Theoretical Concentration (U/mL)	Experimental Concentration (U/mL)	CV (%)*	R
1	8.247	8.48	6%	0.99
2	16.49	17.83	3%	
3	32.99	31.18	8%	
4	49.48	49.51	5%	
5	65.98	59.84	6%	
6	82.47	84.21	1%	

*This CV represents the CV for the replicates of the experimental concentration.

Intra-Assay Precision
A low, medium, and high concentration sample was run with 8 replicates each to evaluate the precision.

Sample	Average Concentration (U/mL)	CV (%)
L	3.77	10%
M	13.83	10%
H	51.83	8%

Inter-Assay Precision
A low, medium, and high concentration sample was run 2 runs a day with 2 replicates in each run for8 days to determine the inter-assay precision.

Sample	Average Concentration (U/mL)	CV (%)
L	3.02	17%
M	12.27	7%
H	48.59	9%

Accuracy
Accuracy was determined after calibration by running two replicates of each control.

Standard	Average Concentration (U/mL)	Target Value ± 15% (U/mL)
3	3.42	2.66 – 3.59
5	13.77	10.63 – 14.38

Hook Effect
No hook effect was observed up to 100 U/mL.

Limit of Blank
The limit of blank (LoB) was determined by 60 replicates of calibrator matrix to be 0.19 U/mL.

Limit of Detection
The limit of detection (LoD) was determined by 60 replicates of low-level samples to be 0.74 U/mL.

Limit of Quantification
The limit of quantification (LoQ) was determined by 60 replicates of low-level samples to be 1.3 U/mL.

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.

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 developed and manufactured by

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

EC	REP

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)