

EDI™ Fecal Clostridium Difficile Toxin A CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the Quantitative Measurement of Clostridium Difficile Toxin A Antigen in Feces.

REF CL0827C IVD 50, 100, 250 +2-8°C

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of Clostridium Difficile Toxin A (*C. Difficile*) antigen levels in feces using the ECL100 or ECL25 Immunoassay analyzer. This test is used in the diagnosis of Clostridium difficile-associated diarrhea and pseudomembranous colitis.

For in-vitro diagnostics purpose only

SUMMARY OF PHYSIOLOGY

Clostridium Difficile is a gram-positive anaerobe. Infection with *C. Difficile* causes severe diarrhea and can be fatal if not diagnosed and treated in a timely manner. *C. Difficile* infection is induced in patients by long-term treatment with antibiotics and is commonly found in hospital environment. It is easily transmitted through contact with infected fecal matter.

Clostridium difficile is a common pathogen and a major cause of infectious diarrhea in hospitalized patients. While most strains produce both Toxin A and Toxin B, some only produce one or the other. These enterotoxins, which are both proinflammatory and cytotoxic, attack the mucosal lining of the intestines. If not identified and treated in a timely fashion, can result in permanent damage to the colon or colitis. This assay employs antibodies specific to Toxin A.

ASSAY PRINCIPLE

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

Assay calibrators, controls, or extracted patient fecal samples are added directly to a reaction vessel. Simultaneously, a biotinylated antibody, a streptavidin coated magnetic particle and subsequently an acridinium ester conjugated antibody are added to the vessel. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of "magnetic particles – biotinylated anti-Toxin A antibody – Toxin A antigen – acridinium ester conjugated anti-Toxin A antigen 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 Toxin A antigen in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in fecal *C. Difficile* Toxin A antigen 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. Toxin A Magnetic Particle Solution (L0584)

Qty: 1 x 2.3 mL (50/kit), 1 x 2.8 mL (100/kit),
1 x 7.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

2. Biotin Toxin A Antibody (L0585)

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

3. Acridinium Ester Toxin A Antibody (L0586)

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. Fecal Toxin A Calibrators (L0589 – L0590)

Liquid Fecal Toxin A in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials 1.0 mL each
Storage: 2 – 8°C
Preparation: Mix by inversions or gentle vortexing.

5. Fecal Toxin A Controls (L0591 – L0592)

Liquid fecal Toxin A in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials 1.0 mL each
Storage: 2 – 8°C
Preparation: Mix by inversions or gentle vortexing.

6. Fecal Extraction Buffer (30669) (Packaged Separately)

Fecal extraction buffer matrix with protein stabilizers and preservative which serves as a ready-to-use patient sample diluent.
Qty: 1 x 180 mL
Storage: 2 – 30°C
Preparation: Ready to 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 (-20°C). If the stool sample is collected and tested in the same day, it is allowed to be stored at 2-8°C.

Patient samples need to be diluted **1:15** with patient sample diluent before being measured. 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 1.5 mL of fecal sample extraction buffer to each tube or vial. Note: Eppendorf Repeater pipette is recommended.
3. With liquid stool sample, using a pair of scissors, cut the pipette tips at a 45-degree angle. Then, use the pipette to transfer 150 µL of the liquid stool sample into the tube or vial above.
4. With solid stool sample, take an equivalent amount (about 100-120 mg, a green pea size) 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 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 from each container on the cartridge.

- 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.
- 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.
 - 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.
- 2.4 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)
Fecal Toxin A Controls (L0591 – L0592)	50	-
Extracted Fecal Samples	-	50
Biotin Toxin A Antibody (L0585)	100	100
Toxin A Magnetic Particle Solution (L0584)	25	25
Acridinium Ester Toxin A Antibody (L0586)	100	100
Incubation Period 1		
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 automatically 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. Control 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. Difficile Toxin A concentrations were measured in stool samples collected from 55 apparently healthy adults using the EDI™ Fecal Clostridium Difficile Toxin A Antigen CLIA Kit. The suggested normal cut off is 0.2 ng/mL.

It is highly recommended that each laboratory should establish their own normal range for fecal Toxin A antigen 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 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 1000 ng/mL.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates of calibrator matrix to be 0.028 ng/mL.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates of low-level samples to be 0.15 ng/mL.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates of low-level samples to be 0.27 ng/mL.

Linearity

Linearity was determined by measuring one diluted standard (L4) of medium Toxin A antigen concentration. In each assay, the average of three replicates of each of the diluted standards is used for a correlation analysis against calculated theoretical values.

Standard L4	Average Measured Concentration (ng/mL)	Theoretical Concentration (ng/mL)	CV (%)	R ²
10%	3.1	2.8	1	0.99
20%	6.3	5.5	6	
40%	12.5	12.3	2	
60%	18.8	16.9	4	
80%	25.0	23.9	3	
100%	31.3	29.4	1	

Repeatability

Reproducibility was determined by measuring ten replicates of two control samples.

Control	Average Concentration (ng/mL)	CV (%)
2	7.71	3%
5	67.82	3%

Precision

Precision was determined by measuring eight replicates of three control samples a total of three times on three different days.

Control	Average Concentration (ng/mL)	CV (%)
2 1:2	3.82	6%
2	7.71	4%
4	29.67	3%

Accuracy

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

Standards	Measured Concentration (ng/mL)	Average Concentration (ng/mL)	Target Value $\pm$ 25% (ng/mL)
L3	15.43	15.91	11.9 – 19.9
	16.55		
	15.75		
L4	19.7	31.31	23.5 – 39.1
	18.4		
	17.9		

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REFERENCES

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Health Protection Agency Collaborating Centre, University College London Hospitals, London, UK
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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)



In Vitro
Diagnostic
Device



European
Conformity



Lot Number



Catalog Number



Read instructions
before use



Number of Tests



Store at



Use by



Keep away from
heat and direct
sun light



Manufacturer



Authorized
Representative
in Europe

WARRANTY