

EDI™ Fecal Clostridium Difficile GDH Antigen CLIA Kit

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

REF CL0824CE IVD   50, 100, 250  

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of Clostridium Difficile GDH (*C. Difficile*) antigen levels in feces using the ECL100 or ECL25 Immunoassay analyzer. This test aids in the screening of *C. difficile* infection.

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.

Since all strains of *C. Difficile* produce large amounts of glutamate dehydrogenase, testing for this antigen has proven to be a better screening tool due to its higher negative predictive value.

ASSAY PRINCIPLE

This CLIA is designed, developed, and produced for the quantitative measurement of GDH antigen in fecal samples. The assay utilizes a two-site "sandwich" technique with two antibodies that bind to different epitopes of GDH 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-GDH antibody – GDH antigen – acridinium ester conjugated anti-GDH 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 GDH 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* 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. GDH Magnetic Particle Solution (L0548)

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 GDH Antibody (L0549)

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 GDH Antibody (L0550)

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 GDH Antigen Calibrators (L0559 – L0560)

Liquid Fecal GDH in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty: 2 x vials of 1.0 mL each

Storage: 2 – 8°C

Preparation: 1.0 mL of Calibrators, mix by inversions or gentle vortexing. Make sure that Calibrators are well mixed before use.

5. Fecal GDH Antigen Controls (L0561 – L0562)

Liquid Fecal GDH in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty: 2 x vials of 1.0 mL each

Storage: 2 – 8°C

Preparation: 1.0 mL of Calibrators, mix by inversions or gentle vortexing. Make sure that Calibrators are well mixed before use.

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 samples should be collected by using a plastic sampling device. The collected fecal sample must be transported, kept at 2-8°C and tested within 2 days. A non-preserved sample must be stored below -20°C for a longer storage period.

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. Centrifuge the diluted fecal sample at 1000 rpm (200 g) for 3 minutes. The supernatant can be directly used in the assay. As an alternative to centrifuging, let the diluted samples sit and sediment for 15 minutes and take the clear supernatant for testing.

*Note: The supernatant **MUST** be particle free, debris free and bubble free before loading to the ECL100 machine for accurate results. If necessary, transfer 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 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)
GDH Antigen Controls (L0561 – L0562)	75	-
Extracted Fecal Samples	-	75
Biotin GDH Antibody (L0549)	100	100
GDH Magnetic Particle Solution (L0548)	25	25
Incubation Period 1		
Wash the reaction cup 3 times with the wash reagent.		
Acridinium Ester GDH Antibody (L0550)	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 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 antigen concentrations were measured in stool samples collected from 45 apparently healthy adults using the EDI™ Fecal Clostridium Difficile GDH Antigen CLIA Kit. The suggested normal cut off is 0.50 ng/mL.

It is highly recommended that each laboratory should establish their own normal range for fecal GDH 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 250ng/mL.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates of calibrator matrix to be 0.46 ng/mL.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates of low-level samples to be 0.51 ng/mL.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates of low-level samples to be 1.54 ng/mL.

Linearity

Linearity was determined by measuring one diluted standard (L6) of medium GDH antigen concentration. In each assay, the average of two replicates of each of the diluted standards is used for a correlation analysis against calculated theoretical values.

Standard L6	Average Measured Concentration (ng/mL)	Theoretical Concentration (ng/mL)	CV (%) Measured Concentration	R ²
10%	7.7	6.25	2%	0.999
20%	14.3	12.5	5%	
40%	30.1	25.0	3%	
60%	44.8	37.5	1%	
80%	58.9	50.0	4%	
100%	76.5	62.5	2%	

Repeatability

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

Control	Average Concentration (ng/mL)	CV (%)
3	5.03	3%
8	157.99	4%

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	2.70	6%
5	19.01	5%

7	82.60	5%
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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)
L2	3.2	3.11	2.1 – 3.5
	2.4		
	3.7		
L5	19.7	18.65	15.1 – 25.1
	18.4		
	17.9		

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. N. Shetty, M. W.D.Wren, P. G. Coen, The journal of Hospital Infection January 2011 Volume 77, Issue 1
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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE












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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

For Reference Use Only