Epitope Diagnostics, Inc.

FOR REFERENCE USE ONLY

EDI™ Fecal C. Difficile GDH ELISA

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Qualitative and semi-Quantitative Determination of C. Difficile Glutamate Dehydrogenase 1 in Feces

REF **KT-828**

Σ 96

+2 +8 °C

For Research Use Only **Not for Use in Diagnostic Procedures**

I. INTENDED USE

This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the qualitative detection of C. difficile glutamate dehydrogenase 1 (GDH) in feces. It is optional to quantify GDH concentrations with this kit. The assay is a useful tool as an aid of detection of C. difficile infection. It is for research use only.

II. SUMMARY OF PHYSIOLOGY

Clostridium difficile is a gram-positive anaerobe. Infection with C. difficlie 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. Accurate testing for toxins proved to be difficult due to assays having low sensitivity. 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.

III. ASSAY PRINCIPLE

This "sandwich" ELISA is designed, developed and produced for the quantitative and qualitative measurement of *GDH* in stool specimen. The assay utilizes the microplate-based enzyme immunoassay technique by coating highly purified antibody onto the wall of microtiter wells.

Assay calibrators/controls and fecal specimen are added to microtiter wells of microplate that was coated with a highly purified monoclonal *anti-GDH* on its wall. During the assay, the GDH Antibody will be bound to the antibody coated plate after an incubation period. The unbound material is washed away and another HRP-conjugated monoclonal antibody which specifically recognizes the protein of *GDH* is added for further immunoreactions. After an incubation period, the immunocomplex of "Anti-GDH Capture Antibody – *GDH* – HRP-conjugated Anti-*GDH* Tracer Antibody" is formed if *GDH* is present in the test sample. The unbound tracer antibody and other proteins in buffer matrix are removed in the subsequent washing step. HRP conjugated tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to *GDH* proteins captured on the wall of each microtiter well is directly proportional to the amount of *GDH* level in each test specimen.

IV. 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.

Prior to use, allow all reagents to equalize to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

1. Anti-GDH Antibody Coated Microplate (Cat. No. 30862)
One microplate with twelve by eight strips (96 wells total) coated with monoclonal anti-human GDH. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

2. Anti-*GDH* Tracer Antibody (Cat. No. 30863)
One vial containing **0.6 mL** horseradish peroxidase (HRP)-conjugated monoclonal *GDH* antibody in a stabilized protein matrix. This reagent should be diluted and stored at 2 – 8 °C and is stable until the expiration date on the kit box.

3. GDH Tracer Antibody Diluent (Cat. No. 30864)
One bottle containing **12 mL** ready-to-use buffer. It should be used only for tracer antibody dilution according to the assay procedure. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

4. ELISA HRP Substrate (Cat. No. 10020)
One bottle containing **12 mL** of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

5. ELISA Stop Solution (Cat. No. 10030)
One bottle containing **12 mL** of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8 °C or room temperature and is stable until the expiration date on the kit box.

6. *GDH* Positive Control (Cat. No. 30865)
1 vial containing **2 mL** of GDH Calibrator 6. This positive control is in a liquid bovine serum albumin-based matrix with non-azide preservative. This is also used as the calibrator 6 for quantitative measurements. **Refer to vial for exact concentration.** This reagent should be stored at 2 – 8 °C and are stable until the expiration date on the kit box.

7. ELISA Wash Concentrate (Cat. No. 10010)
One bottle containing **30 mL** of 30-fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide and non-mercury based preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

8. Concentrated GDH Fecal Sample Extraction Buffer (Cat. No. 30820)
One bottle containing **10 mL** of 10-fold concentrated fecal sample extraction buffer. This reagent should be diluted with **90 mL** distilled water and mixed well. This yields as the fecal sample extraction buffer, calibrator diluent, calibrator 1, and negative control. The Fecal Sample Extraction Buffer may be

stored at 2-8ºC and is stable until the expiration date on the kit box.

V. SAFETY PRECAUTIONS

The reagents must be used in a laboratory and are for professional use only. Materials sourced for reagents containing bovine serum albumin were derived in the contiguous 48 United States and 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 are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. 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.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

- Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1000 µL, etc.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
- Disposable plastic 1000 mL bottle with cap.
- Aluminum foil.
- Deionized or distilled water.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

VII. SPECIMEN COLLECTION & STORAGE

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 1-2g 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 the same day, it is allowed to be stored at 2-8ºC. Avoid more than 3x freeze and thaw.

VIII. ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior use. Please see REAGENTS section for details.

(3) Concentrated Fecal Extraction Buffer must be diluted to working solution prior use. Please see REAGENTS section for details.

(4) **For semi-quantitative measurement:** Prepare **1:2** serially diluted calibrators using GDH Calibrator 6 (30865) and working **(1x) Fecal Extraction Buffer** as the dilution buffer to obtain calibrators 2-5. Store at 2-8 ºC. *Do not freeze*. See example below:

Calibrator	Calibrator Volume	Volume of Fecal Extraction Buffer
CAL 6	*Level : Cat 30865*	-
CAL 5	1 mL of Cal 6	1 mL
CAL 4	1 mL of Cal 5	1 mL
CAL 3	1 mL of Cal 4	1 mL
CAL 2	1 mL of Cal 3	1 ml
CAL 1	*Fecal Extraction Buffer (1x)*	-

2. Patient Sample Preparation

2.1. For manual weighing procedure only:

Patient samples need to be diluted **1:5** with GDH Fecal Extraction Buffer before being measured.

(1) Label a test tube (12x75 mm) or a 4 ml plastic vial.

(2) With solid stool sample, take or weigh an equivalent amount (**about 250mg or 350µL for liquid feces)** with a spatula or a disposable inoculation loop. Suspend the solid/liquid stool sample with **1 mL Fecal Extraction Buffer** and mix well on a vortex mixer.

(3) Centrifuge the diluted fecal sample at 3000 rpm (800-1500 g) for 5-10 minutes. The supernatant can be directly used in the assay. As an alternative to centrifuging, let the diluted samples sit and sediment for 30 minutes and take the clear supernatant for testing.
Note: If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample**.**

(4) This sample can be stored at 2-8ºC up to three (3) days and below -20ºC for longer storage. Avoid more than 3x freeze and thaw cycle.

2.2. Using EDI Fecal Sample Collection Devices (Cat. KT889)

(1) Label a Fecal Sample Collection tube

(2) Follow the instructions on the Sample Collection Tube insert, KT889.

(3) This sample can be stored at 2-8ºC up to three (3) days and below -20ºC for longer storage. Avoid more than 3x freeze and thaw cycle.

(4) Two drops of the extracted sample is equivalent to 100 µl.

3. Assay Procedure

3.1 QUALITATIVE MEASUREMENT (Recommended)

(1) Use the working (1x) fecal extraction buffer (Cat# 30689) as the negative control

(2) Place a sufficient number of *GDH* monoclonal antibody-coated microwell strips (Cat. 30862) in a frame.

(3) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	NEG CTL	SAMPLE 3	SAMPLE 7
B	NEG CTL	SAMPLE 3	SAMPLE 7
C	POS CTL	SAMPLE 4	SAMPLE 8
D	POS CTL	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	SAMPLE 9
F	SAMPLE 1	SAMPLE 5	SAMPLE 9
G	SAMPLE 2	SAMPLE 6	SAMPLE 10
H	SAMPLE 2	SAMPLE 6	SAMPLE 10

(4) Add **100 µL** of controls and extracted patient stool samples into the designated microwell. Mix by gently tapping the plate. Cover the plate with one plate sealer. Cover with foil or other material to protect from light.
Note: *if the collection tubes from KT-889 is used, add two drops of extracted fecal sample into each well.*

(5) Incubate plate at room temperature, static, for **1 hour**.

(6) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(7) Prepare GDH Tracer antibody working solution by **1:21** fold dilution of the antibody (Cat. 30863) with the Tracer Antibody Diluent (Cat. 30864). For each strip, mix **1 mL** of the Tracer Antibody Diluent with **50 µL** of the Tracer antibody in a clean test tube.
(8) Add **100 µL** diluted anti- GDH Tracer Antibody to each well. Mix by gently tapping the plate.
(9) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(10) Incubate plate at room temperature, static, for **30 minutes**.
(11) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(12) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(13) Cover the plate with a new plate sealer and also with aluminum foil to avoid exposure to light.
(14) Incubate plate at room temperature for **15 minutes**.
(15) Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(16) Read the absorbance at 450 nm.

3.2 QUANTITATIVE MEASUREMENT (optional)

(1) Place a sufficient number of *GDH* monoclonal antibody-coated microwell strips (Cat. 30862) in a frame.
(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	CAL 1	CAL 5	SAMPLE 3
B	CAL 1	CAL 5	SAMPLE 3
C	CAL 2	CAL 6	SAMPLE 4
D	CAL 2	CAL 6	SAMPLE 4
E	CAL 3	SAMPLE 1	SAMPLE 5
F	CAL 3	SAMPLE 1	SAMPLE 5
G	CAL 4	SAMPLE 2	SAMPLE 6
H	CAL 4	SAMPLE 2	SAMPLE 6

(3) Add **100 µL** of calibrators and extracted patient stool samples into the designated microwell. Mix by gently tapping the plate. Cover the plate with one plate sealer. Cover with foil or other material to protect from light.
Note: *if the collection tubes from KT-889 is used, add two drops of extracted fecal sample into each well.*
(4) Incubate plate at room temperature, static, for **1 hour**.
(5) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(6) Prepare GDH Tracer antibody working solution by **1:21** fold dilution of the antibody (Cat. 30863) with the Tracer Antibody Diluent (Cat. 30864). For each strip, mix **1 mL** of the Tracer Antibody Diluent with **50 µL** of the Tracer antibody in a clean test tube.
(7) Add **100 µL** diluted anti- GDH Tracer Antibody to each well. Mix by gently tapping the plate.
(8) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(9) Incubate plate at room temperature, static, for **30 minutes**.
(10) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(11) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(12) Cover the plate with a new plate sealer and also with aluminum foil to avoid exposure to light.
(13) Incubate plate at room temperature for **15 minutes**.
(14) Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(15) Read the absorbance at 450 nm.

IX. PROCEDURAL NOTES

1. It is recommended that all calibrators/ controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

X. INTERPRETATION OF RESULTS

QUALITATIVE MEASUREMENT:

1. Calculate the average absorbance for each pair of duplicate test results.
2. Calculate the cut-off
   The positive cut-off and the negative cut-off are established by using following formula.

   Positive Cut-Off = 1.1 x (mean extinction of negative control + 0.10)
   Negative Cut-Off = 0.9 x (mean extinction of negative control + 0.10)

3. Interpret test result
   - Positive: patient sample extinction is greater than the Positive Cut-Off
   - Negative: patient sample extinction is less than the Negative Cut-Off
   - Equivocal: patient sample extinction is between the Positive Cut-Off and the Negative Cut-Off.

4. Assay quality control
   1. Positive control must show an average OD reading greater than 1.2.
   2. Negative control should show an average OD reading less than 0.18

QUANTITATIVE MEASUREMENT:

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the calibrator 1(0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibrator curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-

point curve. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

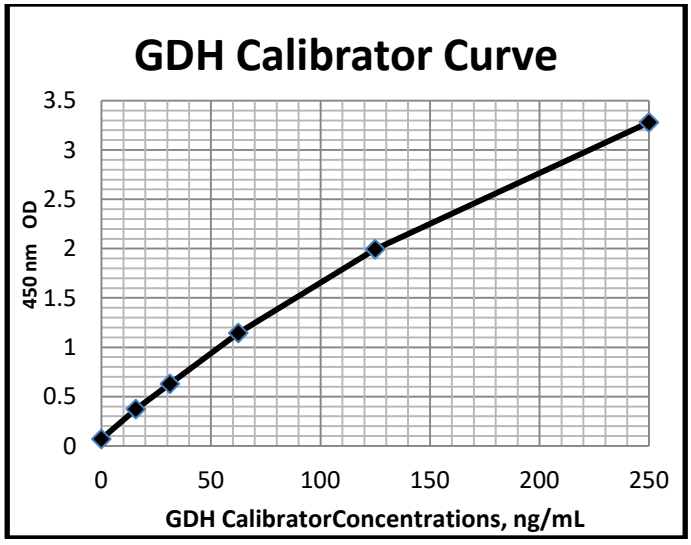
(2) 4. The GDH concentrations for the unknown samples are read directly from the calibrator curve using their respective corrected absorbance.

XI. EXAMPLE DATA AND CALIBRATOR CURVE

QUANTITATIVE MEASUREMENT:

A typical absorbance data and the resulting standard curve from Fecal GDH ELISA are represented. **This curve should not be used in lieu of calibrator curve run with each assay.**

Well I.D.	OD 450 nm Absorbance		
	Readings	Average	Corrected
0 ng/mL	0.074 0.066	0.070	0.000
15.7 ng/mL	0.380 0.367	0.373	0.303
31.3 ng/mL	0.631 0.628	0.629	0.559
62.5 ng/mL	1.132 1.156	1.144	1.074
125.0 ng/mL	2.027 1.961	1.994	1.924
250.0 ng/mL	2.956 3.603	3.279	3.209



QUALITATIVE MEASUREMENT:

A typical absorbance data and the resulting negative control and positive controls are represented. This absorbance must not be used in lieu of control values run with each assay.

	OD 450 nm	Average OD 450 nm
Negative Control	0.074 0.076	0.075
Positive Control	2.921 2.850	2.886

ROW	STRIP 1 (OD 450 nm)	
A	Neg. Ctr	0.074
B	Neg. Ctr	0.076
C	Pos. Ctr.	2.921
D	Pos. Ctr.	2.850
E	Sample 1	0.052
F	Sample 2	0.175
G	Sample 3	0.215
H	Sample 4	0.569

1. The OD of negative controls and positive control meet the Internal Quality Control Standard. The Assay is valid.

2. Calculate the Mean OD for negative control:

   $Mean_{neg.} = (0.074 + 0.076)/2 = 0.075$

3. Calculate the Positive and Negative Cut-Off Value:

Positive Cut-Off = 1.1 x (0.075 + 0.10) = 0.1925
Negative Cut-Off = 0.9 x (0.075 + 0.10) = 0.1575
Equivocal = 0.1574 ~ 0.1924

4. Interpret the Sample Result:

Sample 1 = 0.052 ≤ Negative COV → *Negative*
Sample 2 = 0.175 ≤Pos. COV; ≥ Neg COV → *Equivocal*
Sample 3 = 0.215 ≥ Positive COV → *Positive*
Sample 4 = 0.569 ≥ Positive COV → *Positive*

XII. EXPECTED VALUES

Stool from 41 normal adults were measured with this ELISA kit. We found that normal people show undetectable GDH antigen in the extracted stool sample according to the sample collection, extraction and assay procedures described in this insert. The suggested positive cut-off for fecal GDH antigen is 5 ng/mL. We recommend that all assays include the laboratory's own controls.

XIII. LIMITATION OF THE PROCEDURE

(1) The results obtained with this *GDH ELISA* Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves without taking other clinical findings such as stomach endoscope and biopsy, etc.

(2) For unknown sample value read directly from the assay that is greater than the highest calibrator, it is recommended to measure a further diluted sample for more accurate measurement.

XIV. QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known *GDH Antibody* levels. We recommend that all assays include the laboratory's own controls.

XV. PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity (LLOD) of the GDH ELISA as determined by the 95% confidence limit on 16 duplicate determination of zero standard is approximately 0.523 ng/mL.

Specificity

The assay does not cross react to the following organisms: Cryptosoridium parvum, Giardia lamblia, rotavirus and adenovirus.

Samples / Epitope's ELISA	True Positive	True Negative	Total
Positive	7	0	7
Negative	0	41	41
Total	7	41	48

Sensitivity: 100% (7/7)
Specificity: 100% (41/41)
Accuracy: 100% (48/48)

Spike Recovery
Two controls and three assay calibrators (31.3, 62.5 and 125 ng/mL) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (ng/mL)	RECOVERY %
Neat A	50.3	-
Cal-3 31.3 ng/mL	39.6	97.0
Cal-4 62.5 ng/mL	52.1	92.4
Cal-5 125 ng/mL	73.0	83.2
Neat B	107.2	-
Cal-3 31.3 ng/mL	55.9	84.5
Cal-4 62.5 ng/mL	77.1	90.9
Cal-5 125 ng/mL	117.4	101.1

Precision
The inter-assay precision is validated by measuring two samples in a single assay with 6 replicate determinations.

Mean Value (ng/mL)	CV (%)
60.3	5.4
137.6	3.3

The intra-assay precision is validated by measuring two samples in duplicate in 12 individual assays.

Mean I Value (ng/mL)	CV (%)
44.5	3.7
87.0	3.7

XVI. 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.

XVII. REFERENCES

1. N. Shetty, M.W.D. Wren, P.G. Coen, The Journal of Hospital Infection January 2011 Volume 77, Issue 1
Health Protection Agency Collaborating Centre, University College London Hospitals, London, UK

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.
www.epitopediagnostics.com

This product is developed and manufactured by
Epitope Diagnostics, Inc.
San Diego, CA 92121, USA

Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
In Vitro Diagnostic Device	Use by
Read instructions before use	Lot No.
EC REP Authorized Representative In Europe	

Short Assay Procedure of Fecal C. Difficile GDH ELISA: QUANTITATIVE AND QUALITATIVE MEASUREMENT

(1) Add **100 µL** of controls (or calibrators) and **100 µL or two drops** of patient samples into the designated microwell.
(2) Mix, cover and incubate the plate at room temperature **NO SHAKING for 1 hour**
(3) Wash each well 5 times.
(4) Add **100 µL** of working Tracer Antibody into the designated microwell.
(5) Mix, cover and incubate the plate at room temperature **NO SHAKING for 30 minutes.**
(6) Wash each well 5 times.
(7) Add **100 µL** ELISA HRP Substrate into each well.
(8) Cover and incubate plate at room temperature for **15 minutes**.
(9) Add **100 µL** of ELISA Stop Solution into each of the wells.
(10) Read the absorbance at OD 450 nm.