Package Insert

Fecal C. Difficile GDH ELISA

Catalog Number: CDF35-K01

For Research Use Only. Not for use in diagnostic procedures.

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INTENDED USE

This Eagle Biosciences Fecal C. Difficile GDH ELISA (enzyme linked immunosorbent assay) Assay Kit is intended for the quantitative and qualitative detection of C. difficile glutamate dehydrogenase 1 (GDH) in feces. The assay is a useful tool as an aid of detection of C. difficile infection. The Eagle Biosciences Fecal C.Difficile GDH ELISA Assay Kit is for Research Use Only and is not intended for diagnostic or therapeutic purposes.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

ASSAY BACKGROUND

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. 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.

ASSAY PRINCIPLE

This Eagle Biosciences "sandwich" Fecal C. Difficile 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.

REAGENT 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 come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

Anti-GDH Antibody Coated Microplate

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.

Anti-GDH Tracer Antibody

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.

GDH Tracer Antibody Diluent

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

HRP Substrate

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.

Stop Solution

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.

GDH Positive Control

1 vial containing 1 mL of GDH Control 6. This positive control is in a liquid bovine serum albumin based matrix with non-azide preservative. This is used as the control 6 qualitative 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.

Wash Concentrate

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.

Concentrated GDH Fecal Sample Extraction Buffer

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 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.

SAFETY PRECUATIONS

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.

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.

SPECIMEN COLLECTION AND STORAGE

Fresh fecal samples 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.

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 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. Prepare 1:2 serially diluted calibrators using GDH Calibrator 6 and working (1x) Fecal Extraction Buffer as the dilution buffer to obtain calibrators 2-5. Store at 2-8 ºC. See example below:

PATIENT SAMPLE PREPARATION

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 250µL 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.

   Following is a detailed sample extraction process.

   (a) Label and tare an empty polypropylene tube together with a inoculation loop

(b) Weight 250-500 mg of stool using the inoculation loop by placing it into the pre-tared tube
(c) Record the net amount of sample and break the inoculation loop; leave the lower part of the loop in the tube.
(d) Add diluted Extraction Buffer (4 parts of the stool volume, 1 g stool = 1 ml) into the tube.

Fecal Sample Weight (mg)	Extraction Buffer Volume (ml)
225-274	1.25
275-324	1.50
325-374	1.75
275-424	2.00
425-474	2.25
475-525	2.50

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.

Using Fecal Sample Collection Devices

1. Label Fecal Sample Collection tube
2. Follow the instructions on the sample collection tube insert
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.

ASSAY PROCEDURE

1. Use the working (1x) fecal extraction buffer as the negative control
2. Place a sufficient number of GDH monoclonal antibody-coated microwell strips 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	SAMEPL 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 collection tubes are 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 with the Tracer Antibody Diluent. 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 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 into each of the wells. Mix gently.
16. Read the absorbance at 450 nm.

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.

INTERPRETATION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results
2. Calculate the cut-off

The positive and negative cut-offs are established by using the 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 results.
    a. Positive: Patient sample extinction is greater than the positive cut-off
    b. Negative: Patient sample extinction is less than the negative cut-off
    c. Equivocal: Patient sample extinction is between the positive cut-off and negative cut-off
4. Assay Quality control
    a. Positive control must show an average OD greater than 1.2
    b. Negative control should show an average OD reading less than 0.18

EXAMPLE DATA

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.**

ROW	STRIP 1 (OD 450 nm)	
A	Neg. CTR	0.098
B	Neg. CTR	0.092
C	Pos. Ctr	2.298
D	Pos. Ctr	2.198
E	Sample 1	0.126
F	Sample 2	0.186
G	Sample 3	0.255
H	Sample 4	0.748

1. The OD of negative controls and positive controls meet the Internal Quality Control Standard, The Assay is valid.
2. Calculate the mean OD for negative control.

    $Mean_{neg}$ = (0.098 + 0.092)/2 = 0.095

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

    Positive Cut-Off = 1.1 x (0.095 + 0.10) = 0.2145

    Negative Cut-Off = 0.9 x (0.095 + 0.10) = 0.1755

    Equivocal = 0.176 ~ 0.214

4. Interpret the Sample Result

    Sample 1 = 0.126 ≤ Negative COV →Negative
    Sample 2 = 0.186 ≤ Pos. COV ; ≤ Neg COV →Equivocal

Sample 3 = 0.255 ≥ Positive COV →Positive
Sample 4 = 0.749 ≥ Positive COV →Positive

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.

Samples / Eagle Bio'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)

LIMITATION OF PROCEDURE

1. The results obtained with this Eagle Biosciences Fecal C. Difficile GDH ELISA Assay 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.

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.

PERFORMANCE CHARACTERISTICS

Sensitivity

A positive control of C. Difficile GDH at a concentration of 250 ng/mL was serially diluted with negative control down to 125 ng/ml, 62.5 ng/ml, 31.3 ng/mL, 15.6 ng/mL, 7.2 ng/mL. All these diluted samples were measured with this ELISA kit. The results showed that 7.2 ng/mL was slightly below the positive cut-off interpretation, while 15.6 ng/mL showed a positive test result. Therefore, the analytical sensitivity of this kit is about 9ng/mL

Specificity

The assay does not cross react to the following organisms: Cryptosporidium Parvum, Giardia lamblia, Rotavirus and adenovirus

Precision

Intra-Assay

The intra-assay precision is validated by measuring two positive samples in a single assay with 8 replicate determinations

OD @ 450nm	CV (%)
0.426	4.0
1.028	3.7

Inter-Assay

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

OD @ 450nm	CV (%)
0.347	8.8
1.946	5.6

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

WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.