Package Insert

Fecal C. Difficile Toxin A & B Qualitative ELISA Assay Kit

Catalog Number:
CDT35-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 4.0 (07 NOV 23)

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INTENDED USE

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

SUMMARY OF PHYSIOLOGY

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 both Toxin A and Toxin B.

ASSAY PRINCIPLE

This Eagle Biosciences Fecal C. Difficile Toxin B ELISA Assay Kit is a "sandwich" ELISA is designed, developed and produced for the qualitative measurement of Toxin A and Toxin B in stool specimen. The assay utilizes the microplate-based enzyme immunoassay technique by coating highly purified antibody onto the wall of microtiter wells. Controls and extracted fecal specimen are added to microtiter wells of microplate that was coated with a highly purified monoclonal anti-Toxin A and Toxin B on its wall. During the assay, the Toxin A and B Antibodies 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 Toxin A & B is added for further immunoreactions. After an incubation period, the immunocomplex of "Anti-Toxin A & B Capture Antibody – Toxin A & B – HRP-conjugated AntiToxin A & B Tracer Antibody" is formed if Toxin B 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 Toxin A & B proteins captured on the wall of each microtiter well is directly proportional to the amount of Toxin A & B level in each test specimen.

REAGENTS

This Eagle Biosciences Fecal C. Difficile Toxin A& B ELISA Assay 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. **Toxin AB Antibodies Coated Microplate**
   Microplate coated with monoclonal anti-Toxin A and Toxin B
   Qty: 1 x 96 microplate
   Storage: 2 – 8 °C
   Preparation: Ready to Use

2. **Anti-Toxin AB Tracer Antibodies**
   HRP-conjugated monoclonal toxin A and B antibodies

Qty: 1 x 12 mL
Storage: 2 – 8 °C
Preparation: Ready to Use

3. ELISA HRP Substrate

Tetramethylbenzidine (TMB) with stabilized hydrogen

Qty: 1 x 12 mL
Storage: 2 – 8 °C
Preparation: Ready to Use

4. ELISA Stop Solution

0.5 M sulfuric Acid

Qty: 1 x 12 mL
Storage: 2 – 8 °C
Preparation: Ready to Use

5. Toxin AB Negative Control

Toxin A and Toxin B negative control in a liquid bovind serum albumin based matrix with a non azide preservative

Qty: 1 x 1.0 mL/vial
Storage: 2 – 8 °C; Store at <-20°C after first use. Do not exceed 3 freeze-thaw cycles
Preparation: Ready to Use

6. Toxin AB Positive Control

Toxin A and Toxin B positive control in a liquid bovine serum albumin based matrix with a non-azide preservative.

Qty: 1 x 1.0 mL/vial
Storage: 2 – 8 °C; Store at <-20°C after first use. Do not exceed 3 freeze-thaw cycles
Preparation: Ready to Use

7. Concentrated Fecal Sample Extraction Buffer

Qty: 1 x 10 mL
Storage: 2 – 8 °C
Preparation: 10X concentrate. The contents must be diluted with 90 mL distilled water and mixed well before use.

8. ELISA Wash Concentrate

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty: 1 x 30 mL
Storage: 2 – 25 °C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

SAFETY PRECAUTIONS

The reagents must be used for research use only. 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 are potential infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. Upon 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, 25 µL, 50 µl, 65 µL, 100 µL, and 1000 µL.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm glass or plastic tubes.
- Disposable plastic 1000 mL bottle with cap.
- Aluminum foil.
- 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 & STORAGE

Fresh fecal sample should be collected into stool sample collection container. It is required to collect a minimum of 1-2 mL liquid stool sample or 1-2 g 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 the same day, it is allowed to be stored at 2-8°C. Avoid more than 3x freeze and thaw.

ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature (20-25°C). 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.

2. Patient Sample Preparation

*Patient samples need to be diluted **1:5** with working Fecal Extraction Buffer (1x) 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.
(4) Note: If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample.
(5) 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.

3. Assay Procedure

(1) Place a sufficient number of microwell strips in a holder to run Toxin AB controls and samples in duplicate.
(2) 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

(3) Add **100 µL** of controls and extracted patient stool samples into the designated microwell. Mix by gently tapping the plate.
(4) Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25°C),** for **60 minutes**.
(5) Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
(6) Add **100 µL** of Toxin AB Tracer Antibody to each well. Mix by gently tapping the plate.
(7) Cover the plate with one plate sealer and with aluminum foil. Incubate plate at **room temperature (20-25**°C), for **30 minutes**.
(8) Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(9) Add **100 µL** of ELISA HRP Substrate into each of the wells. Mix by gently tapping the plate.
(10) Cover the plate with a new plate sealer and with aluminum foil. Incubate plate at **(20-25**°C) for **20 minutes**.
(11) Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution into each of the wells. Mix by gently tapping the plate.

(12) Read the absorbance at **450 nm** within **10 minutes** with a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all control 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. Store any unused antibody coated strips in the foil zip-seal bag with desiccant to protect from moisture. Exposure of the plates to humidity drastically reduces the shelf life.
3. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
4. Incubation times or temperatures other than those stated in this insert may affect the results.
5. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate readings.
6. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

INTERPRETATION OF RESULTS

Visual:

1. Positive or reactive: Any sample well that is obviously more yellow than the negative control well.
2. Negative or non-reactive: Any sample well that is not obviously more yellow than the negative control well.

Note: The negative control, as well as some patient samples, may show some slight yellow color. A sample well must be obviously darker or more yellow than the negative control well, when it is interpreted as a positive result.

ELISA Reader:

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
   - Label a test tube (12x75mm) or a 4 mL plastic vial
   - 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 0.6.
    2. Negative control should show an average OD reading less than 0.18.

EXAMPLE DATA AND CALCULATED CUT-OFF

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.

ROW	STRIP 1	(OD 450 nm)
A	Neg. Ctr	0.051
B	Neg. Ctr	0.056
C	Pos. Ctr.	0.892
D	Pos. Ctr.	0.931
E	Sample 1	0.121
F	Sample 2	0.150
G	Sample 3	0.174
H	Sample 4	0.458

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:

   $Mean_{neg.} = (0.051 + 0.056)/2 = 0.053$

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

   Positive Cut-Off = 1.1 x (0.053 + 0.10) = 0.168
   Negative Cut-Off = 0.9 x (0.053 + 0.10) = 0.138
   Equivocal = 0.139 ~ 0.167

4. Interpret the Sample Result:

   Sample 1 = 0.121 ≤ Negative COV → Negative
   Sample 2 = 0.150 ≤Pos. COV; ≥ Neg COV → Equivocal
   Sample 3 = 0.174 ≥ Positive COV → Positive
   Sample 4 = 0.458 ≥ Positive COV → Positive

LIMITATION OF THE PROCEDURE

(1) The results obtained with this Eagle Biosciences Toxin AB ELISA Assay Kit serve only for research use and should not be interpreted as diagnostic in themselves without taking other clinical findings such as endoscopy and biopsy, etc.

QUALITY CONTROL

To assure the validity of the results each assay must include both negative and positive controls. For a valid test, the positive control must have an absorbance of at least 0.6 OD units and the negative control must be less than 0.18 OD units. We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

SENSITIVITY AND SPECIFICITY

Forty stool samples were collected from normal subjects ages 23 to 64 years old. Ten fecal samples from patients with C. difficile infection (also positive in C. difficile GDH by using Clostridium Difficile GDH ELISA Assay Kit) were also collected. All fecal samples were prepared and assayed according to the procedures described in this manual. The results are summarized below.

Test Reslut	Normal Subjects (True Negative)	Patients (True Positive)
Negative	40	3
Positive	0	7
Total	40	10

The sensitivity is 70% (7/10 x 100% = 70%)

The specificity is 100% (40/40 x 100% = 100%)

PERFORMANCE CHARACTERISTICS

Sensitivity

A positive control of C. difficile toxin AB at a concentration of 200 ng/mL was serial diluted with negative control down to 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL and 6.25 ng/mL. All these diluted samples are measured with this kit. The results showed that 12.5 ng/mL had a negative interpretation, while 25 ng/mL showed a positive test result. Therefore, the analytical sensitivity of this kit is about 25 ng/mL.

Specificity

The assay does not cross react to the following:
Helicobacter pylori, glutamate dehydrogenase (GDH) Cryptosoridium parvum, Giardia lamblia, rotavirus and adenovirus.

Reproducibility and Precision

The reproducibility of this assay is validated by measuring two samples both in a single assay of 16-replicate determinations (intra-assay) and in 10 different assays run on different dates (interassay).

Intra-Assay		
	Sample 1	Sample 2
Mean	0.478	0.788
Std Dev	0.047	0.074

CV	9.8%	9.4%
Inter-Assay		
	Sample 1	Sample 2
Mean	0.504	0.827
Std Dev	0.043	0.078
CV	8.5%	9.5%

Interference

Two positive samples are spiked with 5% volume of interference materials to reach a final concentration shown in the table below. All samples are tested in an assay in duplicate.

	Mean OD 450 nm			
	Additive	**Amt Added (mg/mL)**	**Sample**	**Sample 2**
1	Test Control	-	0.352	0.235
2	Bilirubin –L	0.4	0.373	0.214
3	Bilirubin - M	2.0	0.427	0.214
4	Bilirubin - H	10.0	0.319	0.205
5	Test Control	-	0.351	0.132
6	Hb - L	0.4	0.300	0.107
7	Hb – M	2.0	0.343	0.134
8	Hb – H	10.0	0.326	0.093
9	Lipid – L	8	0.304	0.121
10	Lipid - M	40	0.331	0.105
11	Lipid -H	200	0.365	0.80

REFERENCES

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2. Kelly, C.P. Immune response to Clostridium difficile infection. Eur J Gastroenterol Hepatol. 1996; 8: 1048–1053
3. Lyerly, David M., Howard C. Krivan and Tracy D. Wilkins. Clostridium difficile: Its disease and toxins.
4. Borriello, S.P., FE. Barclay, P.J. Reed, A.R. Welch, J.D. Brown, and O.W. Burden. Analysis of latex agglutination test for Clostridium difficile toxin A(D-1) and differentiation between Clostridium difficile toxins A and B, and latex reactive protein. J. Clin Path. 1987. 40:573-580.

SHORT ASSAY PROTOCOL

1. Add **100 uL** of controls and extracted patient stool samples into the designated microwells.
2. Incubate at **room temperature (20-25°C)** for **60 minutes.**
3. Wash each well five times.
4. Add **100 uL** of Toxin AB Tracer Antibody to each well.
5. Incubate at room **temperature (20-25°C**) for **30 minutes.**
6. Wash each well five times.
7. Add **100 uL** of ELISA HRP Substrate into each of the wells.
8. Incubate at **room temperature (20-25°C)** for **20 minutes**.
9. Add **100 uL** of ELISA Stop Solution into each of the wells.
10. Read the absorbance at **450 nm** within **10 minutes** with a microplate reader.

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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.