Epitope Diagnostics, Inc.

EDI™ Fecal E. coli 0157 ELISA

Enzyme Linked Immunosorbent Assay (ELISA) for the Qualitative Determination of Escherichia coli 0157 in Feces

REF **KT-837** EU: IVD CE Σ 96 +2 +8 °C

INTENDED USE

This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the qualitative detection of E. Coli 0157 in feces. It is for in vitro diagnostic use.

SUMMARY OF PHYSIOLOGY

Most *E. coli* strains harmlessly colonize the gastrointestinal tract of humans and animals as a normal flora. However, there are some strains that have evolved into pathogenic *E. coli* by acquiring virulence factors through plasmids, transposons, bacteriophages, and/or pathogenicity islands.

Enterohemorrhagic *Escherichia coli* O157:H7, also known as EHEC, is a major foodborne pathogen causing severe disease in humans worldwide. Healthy cattle are a reservoir of *E. coli* O157:H7, and bovine food products and fresh produce contaminated with bovine waste are the most common sources for disease outbreaks in the United States. *E. coli* O157:H7 also survives well in the environment.

E. Coli O157:H7 is the most predominant strain of Shiga Toxin producing E. Coli in the United States, Japan, and the United Kingdom. The Centers for Disease Control and Prevention (CDC) has estimated that *E. coli* O157:H7 infections cause 73,000 illnesses, 2,200 hospitalizations, and 60 deaths annually in the United States. Healthy adults usually recover from infection with E. coli O157:H7 within a week, but young children and older adults have a greater risk of developing a life-threatening form of kidney failure called hemolytic uremic syndrome

ASSAY PRINCIPLE

This "sandwich" ELISA is designed, developed and produced for the qualitative measurement of E.coli 0157 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 purified monoclonal anti-E. coli 0157 on its wall. During the assay, the E. coli 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 E. coli 0157 is added for further immunoreactions. After an incubation period, the immunocomplex of "Anti-E. coli 0157 Capture Antibody – E.coli – HRP-conjugated Anti-E.coli Tracer Antibody" is formed if E. coli 0157 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 E. coli 0157 proteins captured on the wall of each microtiter well is directly proportional to the amount of E. coli 0157 level in each test specimen.

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

1. E.coli 0157 Antibody Coated Microplate (31003)
One microplate with twelve by eight strips (96 wells total) coated with monoclonal anti-E.coli 0157. 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-E.coli 0157 HRP Conjugate (Cat. No. 31004)**
One vial containing 12 mL ready-to-use horseradish peroxidase (HRP) conjugated monoclonal E.coli antibody in a stabilized protein matrix. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

3. ELISA HRP Substrate (10020)
One bottle contains **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.

4. ELISA Stop Solution (10030)
One bottle contains **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.

5. E. coli Negative Control (31005)
One vial contains E. coli negative control in a liquid bovine serum albumin based matrix with a non-azide preservative. This reagent should be stored at -20°C or below for long- term storage.

6. E. coli Positive Control (31006)
One vial contains E. coli positive control in a liquid bovine serum albumin based matrix with a non-azide preservative. This reagent should be stored at -20°C or below for long- term storage.

7. **Concentrated 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 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.

8. ELISA Wash Concentrate (10010)
One bottle contains **30** mL of 30-fold concentrate. Before use the content 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 preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in the sealed pouch to minimize exposure to air.

SAFETY PRECAUTIONS

The reagents must be used in research laboratory and are for research use only. Reagents containing bovine serum were derived in the contiguous 48 United States and have been 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 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. Upon contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 50 µl, 65 µL, 100 µL, and 1000 µL.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with cap.
6. Aluminum foil.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SPECIMEN COLLECTION

This assay requires stool sample to be collected and diluted with extraction buffer prior to performing the test.

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.

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 250 mg or 250 µL 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 2-8 ºC 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 E. coli 0157 monoclonal antibody-coated microwell strips (Cat. 31003) in a frame.
(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. Cover the plate with one plate sealer. Cover with foil or other material to protect from light.
(4) Incubate plate at room temperature, static, for **1 hour**.
(5) Remove the aluminum foil and 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) Add **100 µL** of E. coli 0157 Tracer Antibody to each well. Mix by gently tapping the plate.
(7) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(8) Incubate plate at room temperature, static, for **30 minutes**.
(9) 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.
(10) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(11) Cover the plate with a new plate sealer and also with aluminum foil to avoid exposure to light.
(12) Incubate plate at room temperature for **20 minutes**.
(13) Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(14) Read the absorbance at 450 nm.

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.

X. 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
   - **i.** Positive: patient sample extinction is greater than the Positive Cut-Off
   - **ii.** Negative: patient sample extinction is less than the Negative Cut-Off
   - **iii.** 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 0.09.

XI. 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	0.049
B	Neg. Ctr	0.048
C	Pos. Ctr.	1.053
D	Pos. Ctr.	1.051
E	Sample 1	0.128
F	Sample 2	0.145
G	Sample 3	0.334
H	Sample 4	0.636

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:

   $Mean_{neg.} = (0.049 + 0.048)/2 = 0.048$

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

Positive Cut-Off = 1.1 x (0.048 + 0.10) = 0.163
Negative Cut-Off = 0.9 x (0.048 + 0.10) = 0.133
Equivocal = 0.134 ~ 0.162

4. Interpret the Sample Result:

Sample 1 = 0.128 ≤ Negative COV → *Negative*
Sample 2 = 0.145 ≤Pos. COV; ≥ Neg COV → *Equivocal*
Sample 3 = 0.334 ≥ Positive COV → *Positive*
Sample 4 = 0.636 ≥ Positive COV → *Positive*

XII. LIMITATION OF THE PROCEDURE

(1) The results obtained with this *E. coli 0157 ELISA* Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves without other clinical findings such as endoscopy and biopsy, etc.

XIII. 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 0.8 OD units and the negative control must be less than 0.09 OD units. We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

XIV. PERFORMANCE CHARACTERISTICS

Specificity

The assay does not cross react to the following:
Toxin A, Toxin B, Helicobacter pylori, glutamate dehydrogenase 1 (GDH), Cryptosporidium parvum, Giardia lamblia, rotavirus and adenovirus.

Precision

Theprecision of this assay is validated by measuring two samples both in a single assay of 16-replicate determinations (intra-assay)

Intra-Assay		
	Sample 1	Sample 2
Mean	1.687	1.975
Std Dev	0.049	0.058
%CV	2.9%	2.9%

Interference

Samples were added 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**

1	Test Control	-	1.938
2	Bilirubin –L	0.4	1.873
3	Bilirubin - M	2.0	1.958
4	Bilirubin - H	10.0	2.023
5	Test Control	-	2.084
6	Hb - L	0.4	2.010
7	Hb – M	2.0	1.936
8	Hb – H	10.0	2.111
9	Lipid – L	8	2.079
10	Lipid - M	40	1.969
11	Lipid – H	200	1.841

	Mean OD 450 nm		
	Additive	**Amt Added (mg/mL)**	**Sample**
1	Test Control	-	0.048
2	Bilirubin –L	0.4	0.045
3	Bilirubin - M	2.0	0.049
4	Bilirubin - H	10.0	0.057
5	Test Control	-	0.047
6	Hb - L	0.4	0.052
7	Hb – M	2.0	0.056
8	Hb – H	10.0	0.053
9	Lipid – L	8	0.048
10	Lipid - M	40	0.050
11	Lipid – H	200	0.047

XV. 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

XVI. REFERENCES

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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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EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

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

Condensed Assay Procedure:

(1) Add **100 µL** of controls or **100 µL or two drops** of extracted patient samples into the designated microwell.
(2) Mix, cover and incubate the plate at room temperature **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 **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 **20 minutes**.
(9) Add **100 µL** of ELISA Stop Solution into each of the wells.
(10) Read the absorbance at OD 450 nm.