Product Manual

E. coli O157 ELISA Kit

Catalog Numbers

BCT-5173 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

E. coli is one of the many groups of bacteria that live in the intestines of healthy humans and most warm-blooded animals. E. coli's serotyping is based on the flagellar (H) and polysaccharide (O) antigens.

Shiga toxin–producing E.coli strains are major foodborne pathogens and have caused a large number of human illnesses. E.coli O157:H7 is the most well-known serotype and it can cause diarrhea, hemorrhagic colitis, and in more severe cases hemolytic uremic syndrome. Most E.coli O157:H7 infection outbreaks have been linked to the consumption of ground beef.

Cell Biolabs' E. coli O157 ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the E. coli O157. The ELISA antibodies specifically recognize the E.coli O157 strain, will not react with other E. coli strains with different O antigens and other types of bacteria such as Shigella sonnei, Pseudomonas aeruginosa and Salmonella flexneri. The ELISA kit has a detection sensitivity limit of 1.2 x 10^4 CFU equivalents/mL E. coli O157. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and food test samples.

Assay Principle

An anti-E.coli O157 monoclonal antibody is adsorbed onto a microtiter plate. E.coli O157 present in the pre-enriched sample or standard binds to the antibody adsorbed on the plate; an FITC-conjugated anti-E.coli O157 monoclonal antibody is added and binds to the E.coli O157 captured by the first antibody. Following incubation and wash steps, a HRP-conjugated mouse anti-FITC antibody is added and binds to the FITC conjugated anti-E.coli O157 monoclonal antibody. Unbound HRP-conjugated mouse anti-FITC antibody is removed during a wash step, and a substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of E.coli O157 present in the sample. The reaction is terminated by addition of Stop Solution and the absorbance is measured at 450 nm. A standard curve is prepared from the provided heat killed E.coli O157 standard and the sample E.coli O157 amount is then determined.

Kit Components

Box 1 (shipped at room temperature)

1. Anti- E.coli O157 Antibody Coated Plate (Part No. 51731B): One strip well 96-well plate.
2. FITC-Conjugated Anti- E.coli O157 Monoclonal Antibody (Part No. 51732C): One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Heat Killed E.coli O157 Standard (Part No. 51733D): One 200 µL vial of 5 x 10^8 CFU equivalents/mL heat killed E.coli O157 in LB broth with 30% Glycerol.

   Note: Before heat inactivation (90-100 ºC for 30 min), the colony forming units (CFUs) of E.coli O157 is predetermined by serial dilution and culture on LB agar plates.

Materials Not Supplied

1. E.coli O157 Sample: Food, Fecal or Environmental Samples
2. Microcentrifuge
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store heat killed E.coli O157 Standard at -80 ºC and avoid freeze/thaw. Store all other components at 4ºC.

Safety Considerations

Remember that your E.coli O157 samples contain potentially pathogenic organisms before heat inactivation; you must follow the recommended NIH and FDA guidelines for all materials containing pathogenic organisms.

Sample Preparation and Enrichment

Homogenize 25 g of samples (ground beef, milk, lettuce, etc) in 225 mL of E.coli O157 culture medium, such as Modified Tryptone Soya Broth with Novobiocin. Incubate 18-24 hrs at 37ºC to 41.5ºC.

Post Enrichment Heat Inactivation

After the enrichment incubation, transfer 1 mL of the culture to a microcentrifuge tube and inactivate the bacteria by heating the sample at 90-100ºC for 30 min. Cool down to room temperature before ELISA steps.

Note: The enriched non-inactivated sample should be kept until the ELISA results are obtained.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- FITC-Conjugated Anti-E.coli O157 Monoclonal Antibody and HRP-Conjugated Anti-FITC Monoclonal Antibody: Immediately before use dilute the FITC-conjugated antibody 1:1000 and HRP-conjugated antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Mix the standard tube well by vortex and prepare a dilution series of the heat killed E.coli O157 Standard in the concentration range of 5 x 10^7 CFU equivalents/mL – 10^4 CFU equivalents /mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	5 x 10^8 CFU Equivalents/mL Heat Killed E.coli O157 Standard (µL)	Assay Diluent (µL)	E.coli O157 (1 x 10^4 equivalent CFUs/mL)
1	40	360	5,000
2	100 of Tube #1	300	1,250
3	100 of Tube #2	300	313
4	100 of Tube #3	300	78
5	100 of Tube #4	300	20
6	100 of Tube #5	300	4.8
7	100 of Tube #6	300	1.2
8	0	300	0

Table 1. Preparation of Heat Killed E.coli O157 Standard

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each E.coli O157 sample, standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of heat inactivated E.coli O157 sample or standard to Anti-E.coli O157 Antibody Coated Plate.
4. Cover with a Plate Cover and incubate at 37ºC for 2 hours.
5. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted FITC-Conjugated Anti-E.coli O157 Monoclonal Antibody to each well.
7. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
8. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 5 above.
9. Add 100 µL of the diluted HRP-Conjugated Anti-FITC Monoclonal Antibody to all wells.
10. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 5 above. Proceed immediately to the next step.

12. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

13. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
14. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical E.coli O157 ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

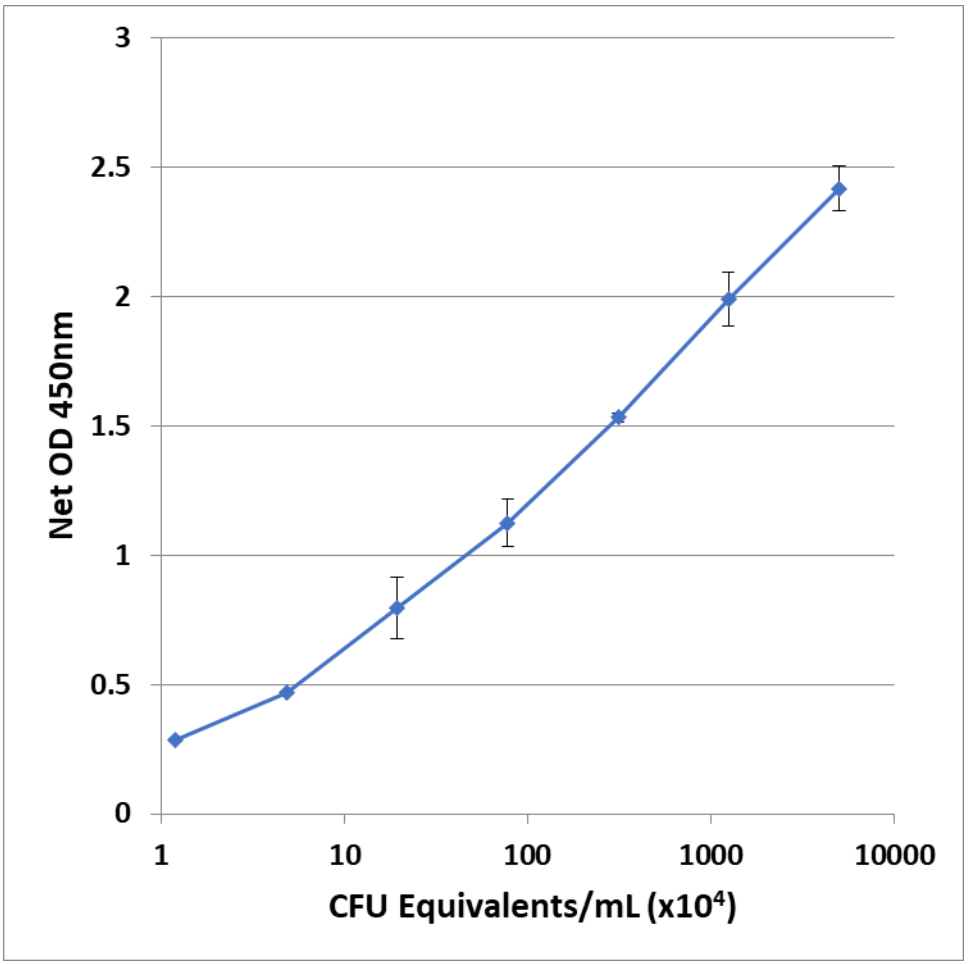

Figure 1: Heat Killed E.coli O157 ELISA Standard Curve.

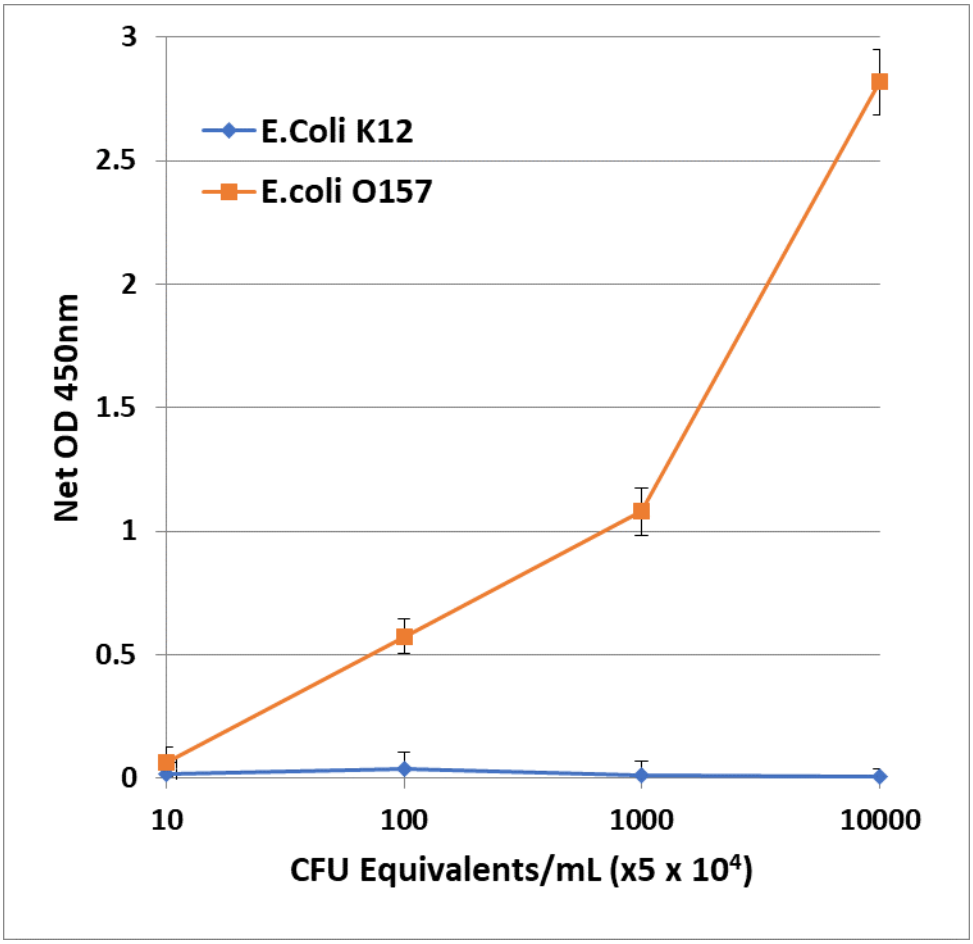

Figure 2: E.coli O157 ELISA Selectivity. Heat killed 5 x 10^8 equivalent CFUs/mL bacterial culture was diluted in Assay Diluent and subjected to E.coli O157 ELISA Kit according to Assay Protocol.

Anti-E.coli O157 Monoclonal Antibody Selectivity	Relative Reactivity
E.coli O157	+++++
Salmonella O30 (hilversum, urbana)	+++++
E.coli O126:H6	-
E.coli O142H6	-
E.coli O116:H10	-
E.coli O119:H27	-
E.coli O20	-
E.coli O125	-
E.coli O55	-
E.coli O111	-
E.coli O7	-
E. hermanii	-
Brucella melitensis	-
Citrobactor freundii	-

Brucella abortus	-
Proteus vulgaris	-
Klebsiella pneumoniae	-
Shigella sonnei	-
Pseudomonas aeruginosa	-
Yersinia enterocolitica	-
Salmonella boydii	-
Salmonella flexneri	-
Salmonelladysenteriae	-

Table 2. E.coli O157 ELISA Specificities.

References

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Warranty

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