E.coli Antigen In Food ELISA test Kit

Cat. No.:DEIA2562
Pkg.Size:96T

Intended use

This assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for the presence of E. coli O157 antigen.

Introduction

Strains of Escherichia coli that produce Shiga-like toxins (SLTs), also known as Verocytotoxins (VTs), are an important cause of human disease. Clinical manifestations of infection include diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Although Shiga-like toxin producing E. coli (SLTEC) belong to many O serogroups, serotypes O157:H7 and O157:H are the predominant SLTEC associated with HC and HUS. Outbreaks of HC and HUS due to E. coli O157 have been linked to consumption of undercooked ground beef. Prevalence studies indicate that up to 3.7% of retail meats may contain E. coli O157. Although this may seem low compared to some other foodborne pathogens, the seriousness of disease caused by E. coli O157 has made this pathogen a major food safety concern. Detection of E. coli O157:H7 in meats by cultural methods is time consuming, requiring several days to obtain presumptive positive results. Typically, meat samples are cultured in primary enrichment broth which is plated onto sorbitol-MacConkey (SMAC) agar. Non-sorbitol fermenting colonies are then subcultured for identification by serotyping or related methods. As some E. coli O157 strains associated with HC and HUS ferment sorbitol, this approach may give false negative results. This ELISA is a rapid and reliable test which significantly reduces the time required to screen foods for the presence of E. coli O157. Primary enrichment cultures grown for 8-16 hours can be tested in less than one hour, allowing ELISA-negative product to be released within 24 hours. Enrichment broths presumptively positive for E. coli O157 on the basis of positive ELISA tests can be cultured further for confirmation by standard methods.

Principle Of The Test

This ELISA is a double antibody (sandwich) ELISA utilizing specific anti-E. coli O157 antibodies coated to microwells. After addition of the sample and the enzyme conjugate, a positive reaction (indicating the presence of E. coli O157 antigen) produces a deep blue color. Addition of the Stop Solution ends the assay and turns the blue color to yellow. The results may be read visually or with an ELISA reader.

Reagents And Materials Provided

1. Microwell test strips containing anti-E. coli O157 polyclonal antibodies: 96 Test Wells
2. Test strip holder: One
3. Enzyme Conjugate: One bottle containing 11 ml of peroxidase conjugated anti-E. coli O157 polyclonal antibody with red dye and a Preservative.
4. Positive control: One vial containing 1 ml of killed E. coli O157 cells in a buffered base.
5. Negative control: One vial containing 1 ml of buffered base.
6. Chromogen: One bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
7. Wash Concentrate 20X: Two bottles containing 25 ml of concentrated buffer and surfactant with preservative.
8. Stop solution: One bottle containing 11 ml of 1 M phosphoric acid.
### Materials Required But Not Supplied

1. Stomacher or blender
2. Shaking or similar incubator
3. Microelisa plate reader capable of reading bichromatically at 450/650 nm (optional)
4. Incubator, 37 °C
5. Pipetter, 100 µl
6. Disposable micropipette tips

**Microbiological media and antibiotics for preparation of necessary enrichment broths and plating media:**

1. Novobiocin
2. Modified EC broth
3. Appropriate containers for storage and disposal of materials potentially contaminated with infectious agents
4. Data record sheets
5. Disinfecting Solution

### Storage

Reagents, strips and bottled components: Store between 2 – 7 °C. Squeeze bottle containing diluted wash buffer may be stored at room temperature.

### Specimen Collection And Handling

#### 1. MEDIUM PREPARATION

Modified EC broth with Novobiocin (mEC+n)

1) Combine the following components with 1 liter of distilled water (if prepared media is not used):

   - Tryptone 20.0 g
   - Lactose 5.0 g
   - K2HPO4 4.0 g
   - KH2PO4 1.5 g
   - NaCl 5.0 g
   - Bile Salt #3 1.5 g

   pH adjusted to 6.9 ± 0.1

2) Autoclave at 121°C for 15 minutes.

3) Allow to cool to room temperature and add 1 ml of a filter sterilized aqueous solution of 20 mg/ml Novobiocin. (for 225 ml add 0.225 ml of Novobiocin solution.) Final concentration should be 20 µg/ml.

#### 2. SAMPLE PREPARATION

1) Add 225 ml of mEC+n to 25 g food product in a sterile stomacher bag or blender jar.

2) Stomach or blend sample and broth for 2 minutes.

3) Transfer stomacher bag to shaker at 37 °C or alternatively transfer contents of blender jar to a sterile flask and attach to shaker.

4) Incubate stomacher bags or flasks at 37 °C with shaking (120 rpm) for 18 hours.

5) Remove a 1 ml aliquot from each sample and place in a separate clean screw top test tube. This is the sample that will be used in the assay.

### Reagent Preparation

**Wash Buffer** - Remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

### Assay Steps

#### TEST PROCEDURE

1. Break off the required number of wells needed (number of samples plus 2) and place in strip holder.
2. Add 2 drops (100 µl) of the negative control to well #1 and 2 drops (100 µl) of the positive control to well #2 (use both as undiluted).
3. Add 2 drops (100 µl) of the test sample to the appropriate well.
4. Incubate at room temperature (15 to 25 °C) for 30 minutes, then wash.
5. Add 2 drops of Enzyme Conjugate (red solution) to each well.
6. Incubate for 30 minutes, then wash*. Rinse wells one time with DI water. Slap out excess fluid against an absorbent towel.
7. Add 2 drops of Chromogen to each well.
8. Incubate for 10 minutes.
9. Add 2 drops of stop solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
10. Read results visually or at 450/620-650 nm.

**NOTE:** Each washing consists of dumping the contents of the wells into an appropriate container with disinfecting solution (e.g. 3% bleach in water) and using the diluted wash buffer to fill to overflowing in each well, shaking out the contents and refilling the wells for a total of 3 times. Samples with sticky particulate matter may require more thorough washing than other samples. The potential exists for false positive results if the sample is not thoroughly washed from the well before addition of subsequent reagents.

Only one set of controls is required per run.
Read results within 4 hours from addition of Stop Solution.
All incubations are at room temperature (15-25 °C)

**Quality Control**

The Positive and Negative Controls must be run each time the assay is performed. For a valid run, the Negative Control must be below 0.12 ODs and the Positive Control greater than 1.0 OD units.

**Interpretation of Results**

1. **INTERPRETATION OF RESULTS - VISUAL**
   Positive: Any sample well that has significant and obvious yellow color. Negative: Any sample well that does not have significant and obvious yellow color.
   **NOTE:** The negative control, as well as some samples, may show some slight color. A sample well must be obviously darker than the negative control well to be called a positive result.
2. **INTERPRETATION OF RESULTS – OD READINGS**
   Positive: OD readings of 0.20 and above.
   Negative: OD readings of less than 0.20.

**Interferences**

No interfering substances which can give erroneous results have been identified. However, media other than those listed must be qualified before use in this ELISA.

**Precautions**

Do not use solutions if they precipitate or become cloudy.
Exception: Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming. Do not add azides to the samples or any of the reagents.
Some reagents contain a preservative.
Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
Limitations

Seeding studies have shown this assay to have a limit of detection of approximately 1,000 CFU/ml, depending on the strain of E. coli O157 tested.
Enrichment is required for 16-18 hours for optimum growth of the E. coli O157. Meats inoculated with less than 1 CFU/g of E. coli O157 were consistently positive in the ELISA after 18 hours of enrichment.

REFERENCES