

Importance of STEC Determination

Shiga toxin-producing *E. coli* (STEC) are food-borne pathogens that cause gastrointestinal illness, hemorrhagic colitis and hemolytic uremic syndrome. *E. coli* O157:H7 is the STEC serotype most often implicated in outbreaks; however, there are numerous other STEC serotypes that have caused serious human illness and outbreaks. STEC that cause human illness were included as notifiable pathogens to the Nationally Notifiable Diseases Surveillance System in 2000. From 2000-2010, 7695 cases were reported; 5688 were associated with the serogroup O157, and the 83% of the other STEC were serogroups with O26, O45, 103, O111, O121 and O145. However, other serogroups, including O91, O113, O104, have also caused serious human. *E. coli* O-157 has been found in ground beef and in cattle hides, and in feces. Bovine feces can be a source of environmental contamination (soil, water) which can lead to secondary contamination of produce growing in fields.

It is difficult to distinguish pathogenic *E. coli* O157 strains from non-pathogenic *E. coli* strains because the former rarely possess any distinguishing phenotypic or biochemical characteristics from the latter. Therefore methods such as the latex agglutination test described in this User's Guide have been developed by the USDA-Agricultural Research Service Eastern Regional Research Center (USDA-ARS-ERRC) to help on the identification of these STEC strains. This latex agglutination method is part of the testing protocol utilized and mandated by the FSIS for testing ground beef and beef trim, and described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5.06 "Detection and Isolation of *Escherichia coli* O157:H7 from Meat Products".

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For ordering or technical assistance contact:

Abraxis LLC
54 Steamwhistle Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

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E. coli O157 IMS Beads

Immunomagnetic Separation Beads for *E. coli* O157



Product No. 543070 (2 mL)

1. General Description

The Abraxis *E. coli* O157 IMS beads are immunomagnetic separation beads designed for the rapid and selective concentration of *Escherichia coli* serogroup O157 to help with their confirmation on selective confirmation agars. The Abraxis *E. coli* O157 IMS beads should be used as part of the USDA-FSIS test protocol described in the USDA-FSIS test protocol described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5.06 "Detection and Isolation of *Escherichia coli* O157:H7 from Meat Products".

2. Safety Instructions

Biological waste should be decontaminated by autoclaving or by using another effective method. Discard samples according to local, state and federal regulations.

3. Storage and Stability

The *E. coli* O157 IMS beads should be stored between 4–8°C (**do not freeze**) until the expiration date as shown on the label. All reagents and samples to be analyzed should be at room temperature before use.

4. Test Principle

The IMS beads provided in the kit are coupled to antibodies against *E. coli* serotype O157. The Abraxis *E. coli* O157 IMS beads should be used as part of the USDA-FSIS test protocol described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5.06 "Detection and Isolation of *Escherichia coli* O157:H7 from Meat Products". The MLG utilizes sample enrichment in modified tryptic soy broth, followed by multiplexed Real Time-PCR assays targeting *stx*₁, *stx*₂ and *eae* genes and the *wxz* gene in the O-antigen gene clusters of the six serogroups, and then immunomagnetic separation (IMS) followed by plating onto Rainbow Agar (mRBA) (Fratamico et al). Colonies are then tested for specific O antigens using latex agglutination and positive colonies are purified on Sheep Blood Agar (SBA) and confirmed using PCR and biochemical identification.

5. Limitations of the Test

If a positive result is obtained on an unknown organism, further test such as PCR should be carried out for confirmation. Apply good judgment to any test result, particularly when preliminary positive results are observed.

6. Warning and Precautions

- This product is for *in vitro* diagnostics use only.
- Do not freeze reagents.
- Do not allow reagents to become contaminated by using dirty transfer pipettes.
- Use reasonable judgment when interpreting the test results.
- Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
- Avoid cross-contamination of samples by using a new sample stick for each sample.
- Specimens may contain pathogenic organisms, handle with appropriate precautions.
- Ensure that reagent bottle caps are tight after each use to prevent drying of reagents.
- Reagents contain 0.05-0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which might cause explosion. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after disposal.
- Dispose according to local regulations.

7. Summary of *E. Coli* O157 in Food Samples Detection Scheme ((MLG) Chapter 5.06)

7.1 Enrichment

325 +/- 32.5 gm sample is combined with 225 +/- 4.5 mL of modified TSB, stomached and incubated static at 42 +/- 1 °C for 15-24 hours. Positive and negative controls need to be included.

7.3 Immunomagnetic Separation (IMS)

Using immunomagnetic separation with specific anti-serotype beads (see protocol below, section D). A post-IMS acid treatment is performed to reduce background flora on the mRBA plate. Following the 1 hour acid treatment, immunomagnetic beads with adhering bacteria (O157) are diluted 1:1 and 1:10. Then, 0.1 mL is spread (plated) onto mRBA. Plates are incubated at 35 +/- 2 °C for 20-24 hours.

7.4 Identification

Colonies are picked from RBA plates and tested for agglutination with antisera specific for the serogroup of interest (at this stage the target serogroup is known). At least one colony of each morphological type on each plate is tested using O157 latex agglutination kits (Abraxis PN 541060). A minimum of five latex positive colonies from each plate are streaked onto SBA plates and incubated at 35 +/- 2 °C for 18-24 hours. Colonies are then confirmed by specific latex agglutination test.

7.5 Confirmation

Latex positive colonies are confirmed by PCR assays followed by biochemical identification.

A. Materials Provided

1. Superparamagnetic beads attached with purified antibodies against *E. coli* O157 covalently bound to the surface. The beads are supplied in a suspension of PBS, pH 7.4 with 0.05% Sodium Azide, 2 mL vial.
2. User's guide.

B. Additional Materials (not provided with the test)

1. Pipettes (10-100 uL)
2. 1 mL Dispenser Pipette
3. mTSB (available from many media manufacturers)
4. Stomacher bags with or without mesh
5. Microcentrifuge tubes (1.5) and 50 mL centrifuge tubes.
6. Test tubes, glassware, loops, and pipettes
7. Wash Buffer Buffer: 0.15M NaCl, 0.01M Sodium Phosphate buffer, pH 7.4, with 0.05% Tween-20. Autoclave at 121 °C for 15 minutes and store refrigerated. Also available in powder form, Sigma product # 740.02. Filter sterilize, pH 7.2 +/- 0.2.
8. Buffered peptone water (BPW). Filter sterilize, pH 7.2 +/- 0.2.
9. E Buffer: to 100 mL of BPW, add and mix 0.5 g of BSA, 50 µL of Tween-20. Filter sterilize, pH 7.2 +/- 0.2.
10. Separation Columns and Separation Magnets (MACS, Miltenyi Biotec) or Multi-6 Magnetic Separator (Abraxis PN 472260)
11. mRBA Plates
12. HCl, 1 N
13. Tube shaker or agitator; Incubator.
14. Vortexer
15. Disinfectant Solution e.g. Sodium hypochlorite solution >1.3% w/w.

C. Test Preparation

1. All reagents and samples to be analyzed should be at room temperature before use.
2. Thoroughly suspend the IMS beads by repeated gentle inversion of the vial.

D. Assay Procedure (Using Abraxis Magnetic Separator*)

Please refer to the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5.06

1. For each sample positive for one of the top six serogroups, transfer 2-5 mL from the overnight enrichment through a 40 µm cell strainer into a 50 mL conical centrifuge tube.
2. Transfer 1.0 mL aliquot of the enrichment filtrate sample to a sterile 1.5 mL centrifuge tube.
3. Add 20 µL of the IMS O157 beads to the sample and vortex for 10-15 seconds to mix.
4. Incubate the sample(s) at room temperature (18-30 °C) with rocking for 15 minutes.
5. Place the sample tube(s) onto the magnetic separation rack. Allow a 3 minute separation.
6. Using a pipette, carefully remove the liquid from the sample tube (**NOTE:** be careful so as not to remove or touch the IMS beads on the side of the tube closest to the magnet).
7. Remove the sample tube(s) from the magnet. Add 1.0 mL of Wash Buffer (E Buffer could also be used) and vortex for 10-15 seconds to re-suspend the IMS beads.
8. Repeat steps 5 to 7, 2 more times (a total of 3 washes).
9. After the final wash step, reconstitute the sample with 1.0 mL of **E Buffer** and vortex 10-15 seconds to mix.
10. Plate 0.1 mL of the bead suspension into mRBA plates. In addition plate 1:10 and 1:100 dilutions of the IMS beads (sample) diluted in **E Buffer**. Use a hockey stick, swab or spreader to spread plate the beads. Incubate for 20-24 hours at 35 +/- 2 °C.
11. **Acid Treatment:** for each sample, transfer 450 µL of the undiluted bead suspension (step 9) to a microcentrifuge tube. Add 25 µL of 1N HCl and vortex for 10-15 seconds (pH should be 2.0-2.5).
12. Place tube(s) in tube rotator and rotate for 1 hour at 18-30 °C.
13. Dilute the IMS bead suspension by adding 475 uL of **E Buffer**.
14. Vortex for 10-15 seconds to re-suspend beads and plate 0.1 mL of the bead suspension into mRBA plates. In addition plate a 1:10 dilution of the IMS beads (sample) diluted in Wash Buffer. Use a hockey stick, swab or spreader to spread plate the beads. Incubate for 20-24 hours at 35 +/- 2 °C.

NOTE: All mixing sticks, tubes, etc. should be sterile.

*Separation Columns and Separation Magnets (MACS, Miltenyi Biotec) can also be used, please refer to procedure described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5.06

E. Identification

Colonies are picked from RBA plates and tested for agglutination with antisera specific for the serogroup of interest (at this stage the target serogroup is known). At least one colony of each morphological type on each plate is tested using O157 latex agglutination kits (Abraxis PN 541070). A minimum of five latex positive colonies from each plate are streaked onto SBA plates and incubated at 35 +/- 2 °C for 18-24 hours. Colonies are then confirmed by specific latex agglutination test.

E. Interpretation of Results/Additional Analysis

Positive samples must be confirmed as described in the USDA-FSIS test protocol described in the USDA-FSIS test protocol described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5.06 "Detection and Isolation of *Escherichia coli* O157:H7 from Meat Products

H. References

(1) Fratamico, P., Bagi, L., Cray, W. Jr., Narang, N., Yan, X., Medina, M., Liu, Y. Detection by Multiplex Real-Time Polymerase Chain Reaction and Isolation of Shiga Toxin-Producing *Escherichia coli* Serogroups O26, O45, O103, O111, O121 and O157 in Ground Beef. Foodborne Pathogens and Disease 8(5):601-607.

(2) USDA-FSIS test protocol described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5.06 "Detection and Isolation of *Escherichia coli* O157:H7 from Meat Products".