applied biosystems by life technologies

Dynabeads® anti-E. coli O157

Catalog nos. 71003, 71004 **Store at 2˚C to 8˚C**

Rev. Date: August 2012 (Rev. 011)

Product Contents

Cat. no.	Volume
71003	1 mL
71004	5 × 1 mL

Product capacity

Capacity for 71003: 50 tests
Capacity for 71004: 250 tests

Dynabeads® anti-E. coli O157 are supplied in a suspension of phosphate buffered saline (PBS) pH 7.4 with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

Intended Use

Dynabeads® anti-E. coli O157 is designed for rapid selective separation of *E. coli* O157:H7 from food, water, or environmental samples. This process can be automated using a BeadRetriever™ benchtop instrument or performed using a manual method. Dynabeads® anti-E. coli O157 are designed for rapid, selective concentration of *E. coli* O157 directly from a pre-enriched sample aliquot using immunomagnetic separation (IMS). Dynabeads® anti-E. coli O157 reacts with all *E. coli* O157 strains including pathogenic and non-pathogenic, sorbitol fermenting and non-sorbitol fermenting isolates. Dynabeads® anti-E. coli O157 are simply incubated with an aliquot of the pre-enriched sample, and the antibodies coated onto the beads will specifically bind the target bacteria. The bead-bacteria complexes are subsequently separated by applying a magnetic field. The whole IMS process can be automated using a BeadRetriever™ instrument or performed manually.

Intended User

Any user who is skilled in using conventional microbiological techniques, equipped, and/or certified to do pathogen testing on food, feed, and environmental samples, may use Dynabeads® anti-E. coli O157. The user must be skilled in using conventional microbiological techniques and in interpreting results.

Sample Matrix

Any food, water, feed, or environmental sample that has been pre-enriched for 6–18 hours in Buffered Peptone Water (BPW), Tryptone Soya Broth (TSB), or Brilliant-Green Bile Broth (BGBB) is suitable for IMS with Dynabeads® anti-E. coli O157.

Interpretation Criteria

The test is based on plating the concentrated bead-bacteria complexes onto internationally accepted *E. coli* O157 culture media, such as Cefixime Tellurite Sorbitol MacConkey agar (CT-SMAC) and CHROMagar® O157. Interpretation of presumptive results depends on the skill of the user to correctly identify and differentiate the isolated colonies based on typical *E. coli* O157 morphology. Suspect colonies must be confirmed by standard biochemical and serological test methods.

Required Materials

For performing automated IMS:

- BeadRetriever™ instrument.
- BeadRetriever™ tubes and tips.

For performing manual IMS:

- Magnets: MPC™-6, MPC™-1, MPC™-S.
- Mixer allowing tilting and rotation of tubes (e.g. MX 1, Sample Mixer).

Other materials:

- CT-supplement.
- CHROMagar® O157.
- Micropipette (10–100 µL).
- 1-mL dispenser pipette.
- Pre-enrichment broths such as BPW, TSB, BGBB, or other pre-enrichment broths.
- Stomacher apparatus and stomacher bag with filter.
- Test tubes, glassware, loops, swabs.
- Washing buffer (PBS Tween®): 0.15 M NaCl, 0.01 M Sodium-Phosphate buffer, pH 7.4, with 0.05% Tween®-20. (Autoclave at 121°C for 15 min, store at 2°C to 8°C.)
- Sorbitol MacConkey agar.

Note: Use analytical grade reagents.

General Guidelines

- Read the instrument operating instructions of the BeadRetriever™ before use.
- To avoid cross-contamination and for safety reasons, perform IMS using the BeadRetriever™. In the absence of the BeadRetriever™, strict adherence to good laboratory practice and the following instructions are a prerequisite to obtaining valid results.

Protocol

The following protocol applies to all samples. Place all of the discarded material in appropriate microbiological containers and autoclave.

Prepare Sample

Food samples

1. Weigh 25 g of food sample and place into a filter homogenizer bag.
2. Add 225 mL of the enrichment medium (e.g. BPW, TSB, or BGBB) and homogenize.
3. Incubate for 6–18 hours at 37°C or 41.5°C.
4. Mix the pre-enriched sample thoroughly by homogenizing once more.

Human stools, bovine feces, and environmental swab samples

1. For human and animal stool samples, prepare a 10% suspension in physiological saline and transfer 1 mL into 10 mL of a suitable enrichment broth.
2. Human rectal swab and environmental swabs samples should be transferred into 10 mL of a suitable enrichment broth.
3. Food samples should also be incubated as described in the preceding guidelines.

Water samples

1. Filter 1 L of water according to standard local procedures.
2. Use flat-ended forceps to remove the filter and transfer directly into a wide-mouthed bottle.
3. Add 90 mL of BPW or TSB to the contents of the bottle and shake vigorously to dislodge bacteria from the surface of the membrane.
4. Incubate for 6–24 hours at 37°C or 41.5°C.
5. The use of a filter aid is recommended for samples that are too turbid for membrane filtration.

Automated Immunomagnetic Separation

Place one disposable sample tube strip into a BeadRetriever™ sample rack for each sample to be processed and, using aseptic technique, dispense reagents into each tube. The tab on the tube strip may be used for labelling samples.

1. Load one BeadRetriever™ sample tube strip for each sample into a sample rack.
2. Resuspend Dynabeads® anti-E. coli O157 until the pellet in the bottom disappears by vortexing.
3. Aseptically add 10 µL of properly mixed Dynabeads® anti-E. coli O157 into the two sample tubes 1 and 2.
4. Aseptically add 500 µL of wash buffer to sample tubes 1 and 2.
5. Aseptically add 1 mL of wash buffer to tubes 3 and 4 within the strip.
6. Aseptically add 100 µL of wash buffer to the 5th tube.
7. Remove the desired tube from the sample rack and place it in a second sample rack one meter away. Add 500 µL of a test sample to tubes 1 and 2 and transfer the inoculated tube back to the first sample rack. Repeat for the remaining samples.
8. Aseptically insert the sterile protective tip combs into the instrument.
9. Insert the rack with filled tubes into the instrument to lock it in place.
10. Check that everything is properly aligned and close the instrument door.
11. Select the EPEC/VTEC program sequence by scrolling with the arrow key and press the START button.
12. While the instrument is in operation, the door must be kept closed. Each processing step and the total time remaining can be followed on the LC display.
13. At the end of the program run, remove the tube's rack from the instrument and plate one half of the bead-bacteria complexes from the 5th tube onto each of the appropriate plating media as recommended in the "Detection & Confirmation of *E. coli* O157" section.
14. Remove the tip combs and discard into a biohazard waste container together with the tube strips.

Manual Immunomagnetic Separation

1. Remove the magnetic plate and load the necessary number of 1.5-mL microcentrifuge tubes into the MPC™-S .
2. Resuspend Dynabeads® anti-E. coli O157 until the pellet in the bottom disappears by vortexing. Pipet 20 µL of Dynabeads® anti-E. coli O157 and dispense into each tube.
3. Add 1 mL of the pre-enriched sample aliquot and close the tube. Change to a new pipette or pipette tip for each new sample.
4. Invert the MPC™-S rack several times. Incubate at room temperature for 10 min with gentle continuous agitation to prevent the beads from settling (e.g. in a MX4 sample mixer).
5. Insert the magnetic plate into the MPC™-S. Invert the rack several times to concentrate the beads into a pellet on the side of the tube. Allow the tube to stand for 3 min for maximum recovery of Dynabeads® anti-E. coli O157.
6. Open the tube cap using the tube opener provided and carefully aspirate and discard the sample supernatant as well as any remaining liquid in the tube cap. (See "Factors Affecting Product Performance".)
7. Remove the magnetic plate from the MPC™-S.

8. Add 1 mL of wash buffer using a different disposable pipette or tip for each sample to prevent cross-contamination between samples as well as the wash buffer. Close the cap and invert the MPC™-S several times to resuspend the beads.
9. Repeat steps 5–8 once.
10. Repeat steps 5–7 once.
11. Resuspend the Dynabeads®-bacteria complex in 100 µL of wash buffer using a different disposable pipette or tip for each sample. Mix briefly by vortexing and proceed to "Detection & Confirmation of *E. coli* O157".

Confirmation

After IMS, transfer the resuspended beads onto each internationally accepted *E. coli* O157 culture media plate. Use two different culture media to increase the chances of detecting suspect colonies that have distinct differential features on each media. We recommend Sorbitol MacConkey (SMAC) media supplemented with CT-supplement and CHROMagar® O157.

1. Spread the bead-bacteria complexes over one half of the plate with a sterile swab to ensure break-up of the bead-bacteria complexes.
2. Dilute further by streaking with a loop. Always carry the loop back into the previously streaked quadrant several times to ensure that the beads reach a fresh, unstreaked quadrant.
3. Incubate the plates at 35°C to 37°C for 18–24 hours. Read the plates for suspect E. coli O157 colorless colonies on CT-SMAC and pink-mauve colored colonies on CHROMagar® O157.

The choice of plating media has been based on some distinct characteristics of *E. coli* 0157:H7. It is the only *E. coli* among clinical isolates which does not ferment sorbitol within 24 hours and which is glucuronidase–negative. The organisms are resistant to potassium tellurite and cefixime. Presumptive *E. coli* O157 colonies must be confirmed by standard biochemical and serological testing.

Specificity And Sensitivity

The protocol for use with Dynabeads® anti-E. coli O157 will determine the presence or absence of one viable *E. coli* O157 in the sample size described if this one cell is able to replicate and is not obstructed by resident background flora. Dynabeads® anti-E. coli O157 will bind both motile and non-motile strains of *E. coli* O157. The binding is independent of the ability to produce either Shiga toxins 1 or 2, or both. Antigenically similar organisms, (e.g. *Escherichia hermannii*, *Salmonella O group N*, or *Proteus spp*.), can crossreact and bind to a limited extent. In addition, extremely "sticky" organisms like *Pseudomonas spp*. or *Serratia* liquefaciens could bind non-specifically. However, the presence of high numbers of competitive background flora in the sample will not affect the binding of *E. coli* O157 to the beads. In naturally contaminated samples the Dynabeads® anti-E. coli O157 protocol, in combination with CT-SMAC agar, can detect *E. coli* O157 from pre-enriched sample aliquots containing as low as 100 *E. coli* O157 cells against high numbers of background flora of 10^6 organisms or more per mL.

False Negatives/Positive Rates

A false negative rate ranging between 2–10% may be expected using the Dynabeads® anti-E. coli O157 protocol depending on the inoculum level, background flora, and sample matrix. However, in identical samples tested without IMS, this false negative rate is significantly increased and is often more than 25%. Therefore, use of the Dynabeads® anti-E. coli O157 protocol will consistently decrease the sample false negative rate by more than 15%. False positives do not occur since all presumptive colonies must always be verified by suitable identification methods. However, the methods depends on the user following good laboratory practices and avoiding cross-contamination of samples. The accuracy of the method is not measurable since IMS is a qualitative, not a quantitative technique. Several bacteria may be bound to the Dynabeads®, but only give rise to one colony-forming unit on the culture media. The precision is dependent on the extent to which particles are recovered from different sample matrices.

Factors Affecting Product Performance

- Perform the IMS procedure on a benchtop at room temperature between 15°C to 25°C using room temperature reagents.
- Ensure that the Dynabeads® are fully dispersed by vortexing >10 sec before use.
- Use filtered pipette tips to transfer samples into the test tubes for manual and automated IMS.
- When performing manual IMS, do not aspirate and discard the isolated bead-bacteria complexes. If the bead-bacteria complexes are aspirated from the sample tube, immediately dispense back into the tube and dilute with wash buffer, then repeat step 5 in the "Manual Immunomagnetic Separation" section before aspirating again.
- In extremely fatty, viscous, or particulate samples, a two-fold sample dilution using the wash buffer must be made prior to IMS to ensure maximum particle recovery.
- During bead-bacteria complex magnetic capture, it is essential with continued gentle rocking of the MPC™-S to prevent binding of magnetic or magnetizable low-mass debris.
- To avoid cross-contamination of the prepared tubes during automated IMS, perform the sample transfer into the tubes in a designated area at least one meter from the prepared tubes.

Description of Materials

Dynabeads® anti-E. coli O157 are uniform, superparamagnetic, polystyrene 2.8 µm beads with adsorbed and affinity-purified antibodies against *E. coli* O157 covalently bound to the surface.

Related Products

Product	Cat. no.
MPC™-1	12001D
MPC™-6	12002D
MPC™-S	A13346
MX1	15907
Sample Mixer	94701
BeadRetriever™	15950
BeadRetriever™ Tubes and Tips	15951

REF on labels is the symbol for catalog number.

References

- Japan - Official Method of the Japanese Health Ministry
- Canada - Health Canada Compendium Official Method – MFLP 90 http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume3/mflp90-01-eng.php

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