applied biosystems
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Dynabeads® EPEC/VTEC O103

Catalog no. 71011 **Store at 2˚C to 8˚C**

Rev. Date: August 2012 (Rev. 003)

Product Contents

Product contents	Volume
Dynabeads® EPEC/VTEC O103	2 mL

Dynabeads® EPEC/VTEC O103 contains a suspension of paramagnetic Dynabeads® specific for the O serogroup O103 of *E. coli*. The beads are suspended in 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

Introduction

Vero cytotoxin-producing *E. coli* serotypes other than O157 VTEC are important human pathogens, and their disease causing abilities as enteropathogenic *E. coli* (EPEC) in animals have been recognized long ago. Non-O157 VTEC infections may be associated with consumption of animal products, although knowledge of their incidence in foods throughout the entire food chain is limited. Some strains of *E. coli* O103 exhibit increased susceptibility to cefixime and tellurite in CT-SMAC and do not seem to grow on this medium. However, Immunomagnetic Separation (IMS) using Dynabeads® EPEC/VTEC O103 represents a physically selective concentration procedure needed to improve the isolation and detection of the organisms from diverse sample matrices. The performance of this product is improved significantly by using the BeadRetriever™, the automated IMS instrument that removes all the major problems associated with manual IMS and assures the safety of test performers.

Intended Use

Dynabeads® EPEC/VTEC O103 is designed for rapid, selective concentration of *E. coli* serotype O103 directly from a pre-enriched sample aliquot using the BeadRetriever™. Dynabeads®, wash buffers, and samples are loaded into the tube-strips provided. All incubations and washing steps are carried out automatically in the instrument. During the incubation process the antibodies coated onto the beads specifically bind the target bacteria. Washing of the beads is achieved by moving the bead-bacteria complexes from tube-to-tube until a final resuspension into the 5th tube for further processing to detect and/or isolate the target organisms.

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® EPEC/VTEC O103. The user must be skilled in using conventional microbiological techniques and in interpreting results.

Sample Matrix

Any food, water, feed, and environmental samples that has been pre-enriched for 24 hours in Buffered Peptone Water (BPW) at 42°C can be used for automated IMS with Dynabeads® EPEC/VTEC O103. Environmental samples include swab streaks of surfaces and containers and fecal material of animal or human origin. A water sample is defined as any source water for potable supply, food is defined as material intended for use in human consumption, and feed is defined as material used for animal consumption.

Interpretation Criteria

Since strains of *E. coli* O103 possess no distinguishing diagnostic feature like sorbitol negativity of *E. coli* O157, no plating medium is particularly recommended except that the medium must be rich enough to allow profuse growth. Modified sorbitol MacConkey agar (CT-SMAC) used with *E. coli* O157 must never be used since some strains of *E. coli* O103 seem to be inhibited on this medium.

Recommended agar/plating media:

- Washed sheep blood agar, or bovine/equine based blood agar with sodium citrate.
- CHROMagar®, MacConkey agar, modified Haemorharragic colitis agar (mHC), Eosin Methylene Blue (EMB), Chromocult® Coliform Agar (Merck).

Follow the swab-streak technique when plating the bead-bacteria complexes as this will result in better isolated colony formation on the culture media. Colonies of presumptive *E. coli* O103 would show the same morphology as any generic *E. coli* on blood agar or any of the previously mentioned plating media. However, these colonies should be serologically confirmed with the agglutination sera recommended for use with the kit and/or by performing other standard differential biochemical tests if necessary.

Required Materials

- 1-mL dispenser pipette.
- BPW (available from most media manufacturers).
- Stomacher and stomacher bag with filter.
- Test tubes, glassware, loops, swabs, pipettes.
- 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.)
- CHROMagar® O157.
- Modified Haemorrhagic Colitis medium (for isolating haemorrhagic colitis strains of *E. coli*) may be prepared from (g/L), tryptone-20; bile salts #3 (1.12), sodium chloride (5), sorbitol (20), bromocresol purple (0.015), distilled water, and Bacto agar (15).
- EPEC/VTEC O103 Antiserum, purchased as Colony Verification Kit from Statens Serum Institut, Denmark.
- All reagents should be of analytical grade.

General Guidelines

- To avoid cross-contamination of the prepared tubes, transfer of sample into the tubes in a designated area at least one meter away from the prepared tubes (see "Automated Immunomagnetic Separation"). Tube-strips for the BeadRetriever™ are designed to fit into the rack in one direction only. Insert tip combs and tube tray as instructed until a click sound is heard. After processing a sample, remove the sample tray first before removing the tip combs. Remove the tip combs at least 10 min after the assay is complete to allow for air-drying before removal.
- For manual IMS, be careful not to aspirate the beads from the sample tube when discarding the supernatant as this results in lack of recovery of *E. coli* O103. If aspiration becomes difficult, leave some of the supernatant in the tube and dilute with wash buffer as this will break the fat content which causes the beads to slide down the tube wall.
- In extremely fatty, viscous, or particulate samples, prepare a two-fold dilution of the sample using the described wash prior to IMS to ensure maximum recovery of particles. Use filtered pipette tips to transfer samples into the test tubes for manual and automated IMS.
- Wear standard laboratory protective clothing.
- Avoid pipetting by mouth.

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 1 bag.
2. Add 225 mL of Buffered Peptone Water (BPW).
3. Incubate at 42°C for 24 hours.
4. Mix the pre-enriched sample thoroughly by homogenizing once more.
5. Using a sterile pipette, transfer a 2 × 0.5 mL or 1-mL aliquot of the filtered suspension to be tested to the assay tubes using immunomagnetic separation (see "Automated Immunomagnetic Separation" and "Manual Immunomagnetic Separation)".

Human Stools, Bovine Faeces, and Environmental Swab Samples

Refrigerate whole stool specimens as soon as possible after collection and examine within 1–2 hours of collection. If they cannot be examined within 1–2 hours, place whole stools or a swab of the stool or rectal swabs in a transport medium (e.g. Stuart's, Cary Blair, etc.) and refrigerate until examination within 2–3 days. If a sample will be held longer than 3 days before examination, freeze at -70°C. Specimens in transport medium should not be left at ambient temperature.

- Transfer 1 mL of human liquid stool sample into 10 mL of BPW.
- For solid human stool samples and bovine faeces, prepare a 10% suspension and transfer 1 mL into 10 mL BPW.
- Human rectal and environmental swab samples should be transferred into 10 mL of BPW.

Human stool, bovine feces and environmental samples must be pre-enriched for 24 hours at 42°C.

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 to the contents of the bottle and shake vigorously to dislodge bacteria from the membrane surface.
4. Incubate at 42°C for 24 hours.
5. The use of a filter aid is recommended for samples that are too turbid for membrane filtration.

Automated Immunomagnetic Separation

All reagents and samples must be aseptically dispensed sequentially into the strips of tubes, after they are fitted into the rack. Users must read the user instructions provided with Dynabeads® EPEC/VTEC O103 before use as follows:

1. Resuspend beads until the pellet in the bottom disappears by using a vortex machine and aseptically add 10 µL into sample tubes 1 and 2.
2. Aseptically add 500 µL of wash buffer to sample tubes 1 and 2.
3. Aseptically add 1 mL of wash buffer to tubes 3 and 4 within the strip.
4. Aseptically add 150 µL of wash buffer to the tube 5.

5. Remove the desired tube from rack A and place in rack B (one meter away). Add 500 µL of a test sample to tubes 1 and 2 and transfer the inoculated tube to rack A. Repeat for the remaining samples.
6. Aseptically insert the sterile protective tip combs into the instrument.
7. Insert the rack containing filled tubes into the instrument, locking it in place.
8. Check that all components are properly aligned and close the instrument door.
9. Select the EPEC/VTEC program sequence by scrolling with the arrow key and press the START button.
10. 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.
11. At the end of the program run, remove the tube rack from the instrument and plate the bead-bacteria complexes from the 5th tube onto the appropriate plating media and proceed to "Culture of *E. coli* O103".
12. Remove the tip combs and discard into a biohazard waste container together with the tube strips.

Manual Immunomagnetic Separation

To avoid cross-contamination and for safety reasons, perform immunomagnetic separation using the BeadRetriever™. In the absence of the BeadRetriever™, strict adherence to good laboratory practice and the following instructions are a pre-requisite to obtaining valid results.

1. Remove the magnetic plate and load the necessary number of 1.5-mL microcentrifuge tubes into the MPC®-S magnet.
2. Resuspend Dynabeads® EPEC-VTEC O103 until the pellet in the bottom disappears by using a vortex machine. Pipet 20 µL of Dynabeads® EPEC-VTEC O103 and dispense into each tube.
3. Add 1 mL of the pre-enriched sample aliquot from "Prepare Samples" and close the tube. Change to a new pipette tip for each new sample.
4. Invert the MPC®-S rack a few times. Incubate at room temperature for 10 min with gentle continuous agitation to prevent the beads from settling (e.g. in a MX1 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 3 min for proper recovery.
6. Open the tube cap using the tube opener provided and carefully aspirate and discard the sample supernatant as well as the remaining liquid in the tube cap.
7. Remove the magnetic plate from the MPC®-S.
8. Add 1 mL of wash buffer (PBS-Tween®). Do not touch the tube with the pipette tip since this can cross-contaminate the samples as well as the wash buffer. Close the cap and invert the MPC®-S a few 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 (PBS-Tween®). Mix briefly using a vortex mixer and proceed to "Culture of *E. coli*".

Culture of *E. coli* O103

After manual or automated IMS, transfer all the resuspended bead-bacteria complex onto blood agar. Alternatively, transfer one half of the bead-bacteria complex onto blood agar and the remaining half onto any one of the following plating media: CHROMagar® O157, MacConkey agar, modified Haemorharragic Colitis agar (mHC), Eosin Methylene Blue (EMB), Chromocult Coliform Agar (Merck).

1. Spread the bead-bacteria complexes over one half of the plate with a sterile swab. This is to ensure the break-up of the bead-bacteria complexes. Dilute further by streaking with a loop. Always carry the loop back into the previously streaked quadrant several times to ensure that the beads are applied to a fresh, unstreaked quadrant.
2. Incubate the plates at 35°C to 37°C for 18–24 hours.
3. Proceed to "Presumptive Identification and Confirmation".

Presumptive Identification and Confirmation

1. Add 10 µL of physiological saline onto a glass slide placed on a dark background. Two or three tests may be performed on one slide.
2. Transfer a sweep of mixed growth from the first half of the blood agar plate onto the slide and make a smooth, milky suspension.
3. Observe for auto-agglutination.
4. In the absence of any auto-agglutination, add 10 µL of the OK O103 antiserum provided in the Colony Verification Kit to the suspension and mix well. Observe for agglutination by tilting the slide for 10–30 sec.
5. If auto-agglutination occurs, test 1–5 distinct individual colonies as described in step 6. A visible agglutination reaction within 30 sec is a strong indication of a presumptive positive sample.
6. When testing the sweep of mixed growth, confirm the initial presumptive result by testing 1–5 distinct individual colonies from the blood agar plate in a similar manner using the OK O103 antiserum. If no distinct colonies could be picked, plate further for purity from the other plating media onto blood agar and proceed as described in this procedure.
7. The reaction is read with the naked eye by holding the slide in front of a light source against a black background (indirect illumination). A positive reaction is seen as a visible agglutination. A negative reaction is persistence of the homogenous milky turbidity.
8. Plate the agglutination positive colonies further for purity and confirm them by standard biochemical, serological, and DNA tests (e.g. PCR).

Specificity and Sensitivity

Following the described protocol for use with Dynabeads® EPEC/VTEC O103 will determine the presence or absence of one viable *E. coli* O103 in the sample sizes described if this one cell is able to replicate and is not obstructed by resident background flora. Dynabeads® EPEC/VTEC O103 will bind both motile and non-motile strains of *E. coli* O103. Antigenically similar (e.g. Escherichia hermannii, Salmonella O group N, or Proteus spp.) can cross-react 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 specific binding of target organisms to the beads. Routinely, immunomagnetically selected and concentrated *E. coli* O103 are detectable on any enteric plating media from pre-enriched sample aliquots containing as little as 100 target cells against high numbers of background flora of 10^6 organisms or more per mL. The accuracy of the method is not measurable since IMS is a qualitative and not a quantitative technique. Several bacteria may be bound to the beads, but only give rise to one colony-forming unit on the culture media. The precision depends on the extent to which particles are recovered from different sample matrices.

False/Negative Rates

Dynabeads® EPEC/VTEC O103 might record a false negative rate ranging between 2–10% depending on the inoculum level, background flora, and sample matrix. In the same sample without IMS, this false negative rate is significantly increased and is often more than 25%. Hence Dynabeads® EPEC/VTEC O103 will consistently decrease the false negative rate by more than 15%. False positives do not occur since the possibility to verify presumptive colonies is always applicable. However the efficacy, of the methods employed depends on the users aptitude in following good laboratory practices and avoiding cross-contamination of samples.

Description of Materials

Dynabeads® EPEC/VTEC O103 are uniform, superparamagnetic, polystyrene microscopic beads with purified antibodies against *E. coli* O103 covalently bound to the surface. Dynabeads® EPEC/VTEC O103 specifically reacts with all strains of *E. coli* O103 serotypes of both human and animal origin.

Related Products

Product	Cat. no.
MPC®-S	A13346
MPC®-1	12001D
MPC®-6	12002D
MX1	15907
BeadRetriever™	15950
CHROMagar® O157	74002

REF on labels is the symbol for catalog number.

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