USER GUIDE

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Dynabeads® MAX *E. coli* O157 Kit

Manual or automated immunomagnetic separation of *E. coli* O157:H7 from food, water, or environmental samples

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For testing of Food and Environmental samples only.

life technologies™

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Product information

Contents and storage

Shipping and storage

All components of the Dynabeads® MAX *E. coli* O157 Kit are shipped at room temperature. Upon receipt, store the components at 2–8°C. If stored properly, all components are guaranteed stable for 12 months *from date of receipt*.

Note: You may store 10X Buffer at room temperature for up to 7 days.

Kit contents

The components supplied in each Dynabeads® MAX *E. coli* O157 Kit are listed below.

Note: The 10X Buffer may be provided in excess of the amount needed.

Component	Cat. no. A10714	Cat. no. A10715
	1-mL kit (50 tests)	5-mL kit (250 tests)
Dynabeads® MAX anti-*E. coli* O157	1 mL	5 × 1 mL
10X Buffer	2 × 8 mL	80 mL

Description of the system

Dynabeads® MAX *E. coli* O157 Kit

E. coli serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related potent toxins that cause severe damage to the lining of the intestine. These toxins (verotoxin, shiga-like toxin) are closely related or identical to the toxin produced by *Shigella dysenteriae*. Typical isolation methods for *E. coli* serotype O157:H7 are both time consuming and can suffer from interference from background microorganisms.

The Dynabeads® MAX *E. coli* O157 Kit utilizes a new, high capacity, anti-*E. coli* O157 particle for rapid selective separation of *E. coli* O157:H7 from food, water, or environmental samples via immunomagnetic separation (IMS). The selectivity of IMS technology improves the quality of sample preparation, simplifies the testing process, and speeds the time to results. This process can be automated using a BeadRetriever™ bench-top instrument, performed using a manual method, or scaled up to handle volumes ranging from 10–50 mL of pre-enriched starting material. A new rapid method is included for isolation of *E. coli* O157 from samples and detection within an 8-hour time period.

The Dynabeads® MAX *E. coli* O157 Kit is designed for use by any laboratory equipped and certified to do pathogen testing on food, feed, and environmental samples. The user should be skilled in using conventional microbiological techniques and interpreting results.

Sample types

The Dynabeads® MAX *E. coli* O157 Kit is designed for testing food, feed, or environmental samples that have been pre-enriched for 6–18 hours in broths such as Buffered Peptone Water (BPW), Tryptone Soya Broth (TSB) or Brilliant-Green Bile Broth (BGBB).

Strains

Dynabeads® MAX anti-*E. coli* O157 beads react with all *E. coli* O157 strains, including pathogenic, non-pathogenic, sorbitol fermenting, and non-sorbitol fermenting isolates.

Continued on next page

Description of the system, continued

Assay principle

The Dynabeads® MAX *E. coli* O157 Kit is designed for the rapid concentration of *E. coli* O157 from pre-enriched samples using IMS. Using the kit, you incubate Dynabeads® MAX anti-*E. coli* beads with an aliquot of pre-enriched sample, and the antibodies coated on the beads specifically bind the target bacteria. The bead-bacteria complexes are subsequently separated from the suspension by applying a magnetic field. The complete IMS process can be performed manually or automated using the BeadRetriever™ system.

Following separation, the concentrated bead-bacteria complexes are ready for plating onto accepted *E. coli* O157 culture media, such as Cefixime Tellurite Sorbitol MacConkey agar (CT-SMAC) and CHROMagar® O157. You can then differentiate the isolated colonies based on typical *E. coli* O157 morphology and confirm suspected colonies by standard biochemical and serological test methods.

Materials

- **Dynabeads® MAX anti-*E. coli* O157** beads are uniform, superparamagnetic, polystyrene microscopic beads with adsorbed and affinity-purified antibodies against *E. coli* O157 covalently bound to the surface. The beads 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 (NaN_3). Sufficient Dynabeads® MAX anti-*E. coli* O157 beads are provided to perform either 50 (1-mL size) or 250 (5-mL size) tests.
- **10X Buffer** is a clear, colorless solution. It may be stored at room temperature for up to 7 days. Equilibrate to room temperature (15–22°C) before use.

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BeadRetriever™ System

The BeadRetriever™ system enables the rapid purification and concentration of selected targets, including *E. coli* O157, from a broad range of samples using Dynabeads® particle-based separation technology. The BeadRetriever™ system allows automated processing of up to 15 samples in ~40 minutes. Simply load each sample and the appropriate reagents into a 5-tube strip, insert the strips in the instrument, and run the program. After processing, the samples are ready to plate.

The BeadRetriever™ system minimizes the risk of cross-contamination and sample handling errors and provides high reproducibility of results.

Additional materials

Additional materials and equipment not supplied with the Dynabeads® MAX *E. coli* O157 Kit (see **Additional products**, page 19):

- Sterile pipettes and filtered pipette tips
- Vortex mixer
- Sterile tubes, glassware, loops, and swabs
- Pre-enrichment broths such as Buffered Peptone Water (BPW), Tryptone Soya Broth (TSB), or Brilliant-Green Bile Broth (BGBB).
- Sorbitol MacConkey (SMAC) agar supplemented with CT-supplement
- CHROMagar® O157 media (see page 19 for ordering information)
- ***Automated protocols:***
  - BeadRetriever™ System
  - BeadRetriever™ Tubes and Tips
  - BeadRetriever™ Tube Rack
- ***Manual protocols:***
  - Magnet capable of holding 1.5-mL tubes, such as the MPC®-S or DynaMag™-2
  - *Optional:* Mixer such as the Dynabeads® MX1 Mixer or Dynabeads® MX4 Mixer

All reagents should be of analytical grade.

Prepare samples

Take all appropriate precautions when handling samples and dispose of all disposable materials in waste containers labeled for biohazardous materials.

Pre-enrichment broths

A variety of broths are available for enriching samples prior to IMS. Based on in-house testing and international regulatory requirements, we recommend the following:

- Buffered Peptone Water (BPW)
- Tryptone Soya Broth (TSB)
- Brilliant-Green Bile Broth (BGBB).

Prepare samples

Food and environmental samples

1. Collect and enrich food and environmental samples according to your standard laboratory procedure.
2. Using a sterile pipette, transfer two 0.5-mL aliquots or one 1-mL aliquot of the sample to the assay tubes that will be used for immunomagnetic separation (see the **Automated isolation protocol** on page 9 and the **Manual isolation protocol** on page 11).

Water samples

Filter 1 liter of water according to standard local procedures. Use flat-ended forceps to remove the filter and transfer directly into a wide-mouthed bottle. 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. Incubate for 6–24 hours at 37°C or 41.5°C. The use of a filter aid is recommended for samples that are too turbid for membrane filtration.

Automated isolation protocol

Introduction

This section contains a procedure for the automated processing of samples using the BeadRetriever™ system (see page 7 for a description). The BeadRetriever™ system enables rapid processing of samples and minimizes sample handling errors.

Additional materials

In addition to the kit components, the following materials are required (see **Additional products** on page 19):

- Sterile pipettes and filtered pipette tips
- Vortex mixer
- BeadRetriever™ System
- BeadRetriever™ Tubes and Tips
- BeadRetriever™ Tube Rack

Prepare beads

Use 20 µL of Dynabeads® MAX anti-*E. coli* O157 beads per sample. Remove Dynabeads® MAX anti-*E. coli* O157 beads from 4°C storage, vortex briefly to mix, aliquot the required volume of beads into a clean tube, and bring to room temperature prior to use.

Prepare 1X Buffer

Prepare ~3.1 mL of 1X Buffer per sample. To prepare 3.1 mL of 1X Buffer, dilute 310 µL of 10X Buffer in 2.79 mL of deionized water.

Important

- Use proper aseptic handling techniques for all steps.
- Use filtered pipette tips for aspirating samples.
- All reagents must be at room temperature (15–25°C) before use.
- Transfer the samples into the tubes in a designated area at least 1 meter from the prepared tubes.
- Replace the cap on each tube before processing the next sample/ tube.

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Automated isolation protocol, continued

Automated isolation protocol

Follow the protocol below to process samples using the BeadRetriever™ system. See the BeadRetriever™ User Manual for complete instrument operating instructions.

1. Place one BeadRetriever™ tube strip into a BeadRetriever™ sample rack for each sample to be processed. The tab on the tube strip may be used to label each sample.
2. Resuspend Dynabeads® MAX anti-*E. coli* O157 beads by vortexing until the pellet is fully dispersed. Make sure the beads are at room temperature.
3. To tubes 1 and 2 in each tube strip, add 10 µL each of Dynabeads® MAX anti-*E. coli* O157 beads.
4. To tubes 1 and 2, add 500 µL each of 1X Buffer.
5. To tubes 3 and 4, add 1 mL each of 1X Buffer.
6. To tube 5, add 100 µL of 1X Buffer.
7. Remove the first tube strip from the sample rack and place it in a second sample rack at least 1 meter away. To tubes 1 and 2, add 500 µL each of the first test sample. Transfer the inoculated strip back to the sample rack. Repeat for the remaining samples.
8. Insert the sterile protective BeadRetriever™ tip combs into the instrument.
9. Insert the rack with filled tubes into the instrument and 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.

    Note: Keep the door closed while the instrument is in operation. Each processing step and the total time remaining can be followed on the instrument display.
12. At the end of the program run, remove the tube rack from the instrument. **The bead-bacteria complexes are in tube 5, and are ready for plating.**
13. Remove the tip combs and discard into a biohazard waste container together with the tube strips.

Manual isolation protocol

Introduction

This section contains a procedure for the manual processing of samples.

Additional materials

In addition to the kit components, the following materials are required (see **Additional products** on page 19):

- Sterile pipettes and filtered pipette tips
- 1.5-mL microcentrifuge tubes
- Magnet capable of holding 1.5-mL tubes, such as the MPC®-S or DynaMag™-2
- Vortex mixer
- *Optional:* Mixer such as the Dynabeads® MX1 Mixer or Dynabeads® MX4 Mixer

Important

- Use proper aseptic handling techniques for all steps.
- Use filtered pipette tips for aspirating samples.
- All reagents must be at room temperature (15–25°C) before use.
- Transfer the samples into the tubes in a designated area at least 1 meter from the prepared tubes.
- Replace the cap on each tube before processing the next sample/ tube.

Prepare 1X Buffer

Prepare 2.1 mL of 1X Buffer per sample. To prepare 2.1 mL of 1X Buffer, dilute 210 µL of 10X Buffer in 2.89 mL of deionized water.

Highly viscous samples

If aspiration becomes difficult, leave some of the supernatant in the tube and dilute with wash buffer. In fatty samples, this will break down the fat content that causes the Dynabeads® beads to slide down the tube wall. In extremely fatty, viscous, or particulate samples, prepare a two-fold sample dilution using the 1X Buffer prior to IMS to ensure maximum particle recovery.

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Manual isolation protocol, continued

Removing liquid from tubes

When removing liquid from the tube containing the beads bound by the magnet, be very careful *not* to aspirate and discard the beads, which contain the bound bacteria. We recommend using a manual pipettor to remove all liquid. Vacuum aspirators are *not* recommended, since they have been shown to reduce the recovery of bacteria.

If the Dynabeads®-bacteria complexes are accidentally aspirated from the sample tube, immediately dispense back into the tube and dilute with wash buffer, then repeat the magnetic capture step (Step 6 of the following protocol).

Manual isolation protocol

1. Remove the magnetic plate from the magnetic capture unit (*e.g.*, the MPC®-S or DynaMag™-2), and insert a 1.5-mL microcentrifuge tube for each sample.
2. Resuspend the Dynabeads® MAX anti-*E. coli* O157 beads by vortexing until the pellet is fully dispersed. Make sure the beads are at room temperature.
3. Add 20 µL of Dynabeads® MAX anti-*E. coli* 0157 beads to each 1.5-mL tube.
4. Add 1 mL of pre-enriched sample to each tube and close the tube. Change to a new pipette for each new sample.
5. Invert the tubes several times, and incubate the samples for 10 minutes with gentle, continuous mixing to prevent the beads from settling.

   Note: We recommend using a mixer such as the Dynabeads® MX1 Mixer or Dynabeads® MX4 Mixer. For manual mixing, invert the sample gently for 15 seconds every minute.
6. Insert the magnetic plate into the magnetic capture unit, and invert the rack several times to concentrate the beads into a pellet on the side of each tube. Allow the tube to stand for 3 minutes for maximum recovery.
7. Open the tube cap and carefully aspirate and discard the sample supernatant as well as any remaining liquid in the tube cap (see **Removing liquid from tubes,** above).

Protocol continued on the next page

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Manual isolation protocol, continued

Manual isolation protocol, continued

Protocol continued from the previous page

8. Remove the magnetic plate from the magnetic capture unit.
9. Add 1 mL of 1X Buffer to each tube using a different disposable pipette or tip for each sample to prevent cross-contamination. Close the cap and invert the rack several times to resuspend the beads.
10. Repeat steps 6–9.
11. Repeat steps 6–8 one more time.
12. Resuspend the Dynabeads®-bacteria complexes in 100 µL of 1X Buffer using a different disposable pipette or tip for each sample.
13. The bacteria-bead complexes are now ready for plating.

Automated isolation protocol—high sensitivity

Introduction

This section contains a procedure for the maximum capture of low cfu/mL samples using the BeadRetriever™ system. Using this protocol, pre-enrichment times can be reduced to 5 hours. Note that using this protocol will reduce the number of tests per kit in half.

Additional materials

In addition to the kit components, the following materials are required (see **Additional products** on page 19):

- Sterile pipettes and filtered pipette tips
- Vortex mixer
- BeadRetriever™ System
- BeadRetriever™ Tubes and Tips
- BeadRetriever™ Tube Rack

Prepare beads

Use 40 µL of Dynabeads® MAX anti-*E. coli* O157 beads per sample. Remove Dynabeads® MAX anti-*E. coli* O157 beads from 4°C storage, vortex briefly to mix, aliquot the required volume of beads into a clean tube, and bring to room temperature prior to use.

Prepare buffers

The High Sensitivity protocol uses both 10X Buffer and 1X Buffer.

Prepare 2.1 mL of 1X Buffer per sample. To prepare 2.1 mL of 1X Buffer, dilute 210 µL of 10X Buffer in 2.89 mL of deionized water.

Important

- Use proper aseptic handling techniques for all steps.
- Use filtered pipette tips for aspirating samples.
- All reagents must be at room temperature (15–25°C) before use.
- Transfer the samples into the tubes in a designated area at least 1 meter from the prepared tubes.
- Replace the cap on each tube before processing the next sample/ tube.

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Automated isolation protocol—high sensitivity, continued

Automated isolation protocol

Follow the protocol below to process samples using the BeadRetriever™ system. Refer to the BeadRetriever™ User Manual for complete instrument operating instructions.

1. Place one BeadRetriever™ tube strip into a BeadRetriever™ sample rack for each sample to be processed. The tab on the tube strip may be used to label each sample.
2. Resuspend Dynabeads® MAX anti-*E. coli* O157 beads by vortexing until the pellet is fully dispersed. Make sure the beads are at room temperature.
3. To tubes 1 and 2 in each tube strip, add 20 µL each of Dynabeads® MAX anti-*E. coli* O157 beads.
4. To tubes 1 and 2, add 100 µL each of 10X Buffer.
5. To tubes 3 and 4, add 1 mL each of 1X Buffer.
6. To tube 5, add 100 µL of 1X Buffer.
7. Remove the first tube strip from the sample rack and place it in a second sample rack at least one meter away. To tubes 1 and 2, add 1 mL each of the first test sample. Transfer the inoculated strip back to the sample rack. Repeat for the remaining samples.
8. Insert the sterile protective BeadRetriever™ tip combs into the instrument.
9. Insert the rack with filled tubes into the instrument and 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.

    Note: Keep the door closed while the instrument is in operation. Each processing step and the total time remaining can be followed on the instrument display.
12. At the end of the program run, remove the tube rack from the instrument. **The bead-bacteria complexes are in tube 5, and are ready for plating.**
13. Remove the tip combs and discard into a biohazard waste container together with the tube strips.

Plate and analyze samples

Plating media

After IMS, transfer each sample onto an internationally accepted *E. coli* O157 culture media plate. We recommend using two different culture media, to increase the chances of detecting suspect colonies that have distinct differential features on each media. We recommend the following (see **Additional products**, page 19):

- Sorbitol MacConkey (SMAC) agar supplemented with CT-supplement
- CHROMagar® O157 media (see page 19 for ordering information)

The choice of plating media is based on some distinct characteristics of *E. coli* 0157:H7. It is the only *E. coli* among clinical isolates that does not ferment sorbitol within 24 hours and that is glucuronidase negative. The organisms are resistant to potassium tellurite and cefixime.

Plate samples

Spread the bead-bacteria complexes over one half of the plate with a sterile swab. This ensures 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 reach a fresh unstreaked quadrant.

Incubate the plates at 35–37°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 media.

Sensitivity and specificity

Strains

Dynabeads® MAX anti-*E. coli* O157 beads react with all *E. coli* O157 strains, including pathogenic, non-pathogenic, sorbitol fermenting, and non-sorbitol fermenting isolates.

The protocol in this manual can 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 competed out by resident background flora. Dynabeads® MAX anti-*E. coli* O157 beads 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 hermanii*, *Salmonella* O group N, or *Proteus* spp.) can cross-react and bind to a limited extent.

In addition, extremely "sticky" organisms such as *Pseudomonas* spp. or *Serratia liquifaciens* 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, IMS with Dynabeads® MAX anti-*E. coli* O157 beads in combination with CT-SMAC agar can detect *E. coli* 0157 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.

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Sensitivity and specificity, continued

False negative/ positive rates

A false negative rate ranging between 2% and 10% may be expected using the Dynabeads® MAX *E. coli* O157 Kit, depending on the inoculum level, background flora, and sample matrix. However, in identical samples tested without IMS, this false negative rate was often more than 25%. Therefore, we found that use of the Dynabeads® MAX *E. coli* O157 Kit consistently decreased 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 method 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 and not a quantitative technique. Several bacteria may be bound to the Dynabeads, but only give rise to one colony-forming unit (cfu) on the culture media. The precision is dependent on the extent to which particles are recovered from different sample matrices.

Appendix A: Ordering information

Accessory products

Additional products

The table below lists additional products that may be used with the Dynabeads® MAX *E. coli* O157 Kit. For more information about these and other microbiology isolation products, refer to **www.lifetechnologies.com/support** or contact Technical Support (page 20).

Product	Description	Catalog no.
DynaMag™-2 Magnet	Magnet capable of holding tubes of 16 × 1.5–2-mL tubes. For use in sample separation using Dynabeads® magnetic beads.	123-21D
MPC®-S	Magnet capable of holding up to six 1.5-ml microcentrifuge tubes. For use in sample separation using Dynabeads® magnetic beads.	A13346
Dynabeads® MX1 Mixer	Holds 12 sample tubes ranging from 1.5-mL microcentrifuge tubes to 1.7 mm in diameter	159-07
Dynabeads® MX4 Mixer	Holds two MPC®-S sample racks; capacity up to 12 microcentrifuge tubes.	159-10
Dynabeads® Rotary Mixer	Contains interchangeable rods to accommodate different tube sizes and sample volumes.	947-01
CT-Supplement	Added to standard Sorbitol MacConkey media (SMAC) to form CT-SMAC. CT-SMAC selects for *E. coli* O157 after IMS.	740-01
CHROMagar® O157	Recommended as a second media to CT-SMAC. CHROMagar® O157 distinguishes between *Pseudomonas* spp. and *E. coli* O157 by a specific color reaction.	740-02
BeadRetriever™ System	Enables rapid, automated purification and concentration of selected targets from a broad range of samples.	159-50
BeadRetriever™ Tubes and Tips	For sample processing using the BeadRetriever™ System. Each tube strip contains five tubes. Disposable plastic tip combs shield the magnetic surface from contact with samples.	159-51
BeadRetriever™ Tube Rack	Contains fifteen separate sample tube slots for use with the BeadRetriever™ System.	159-52

Documentation and support

Obtaining support

Safety Data Sheets (SDS)	Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.
Certificate of Analysis	The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.
Technical Support	For the latest services and support information for all locations, go to **www.lifetechnologies.com/support**. At the website, you can: • Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities • Search through frequently asked questions (FAQs) • Submit a question directly to Technical Support (**techsupport@lifetech.com**) • Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents • Obtain information about customer training • Download software updates and patches
Food Safety support	Website: **www.lifetechnologies.com/foodsafety** Support email: **foodsafety@lifetech.com** Phone number in North America: 1-800-500-6855 Phone number outside of North America: Visit **www.lifetechnologies.com/support**, select the link for phone support, and select the appropriate country from the dropdown menu.

Obtaining support, continued

Limited product warranty	Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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