U.S. English

Product Number:
9755

Reveal®

for E. coli O157:H7

AOAC Official Methods of Analysis℠
2000.13
2000.14

NEOGEN®

Reveal® for *E. coli* O157:H7

Product Number: 9755

Intended Use

The Reveal® for *E. coli* O157:H7 test system provides for the rapid recovery of *Escherichia coli* O157:H7 organisms in various foods, allowing detection and presumptive identification of the test organism in as little as 8 hours.

In an AOAC Official Methods℠ study (2000.13), the Reveal for *E. coli* O157:H7 8-hour test system was found to be an effective procedure for the detection of *E. coli* O157:H7 in raw ground beef, raw beef cubes, and iceberg lettuce rinse. In a second AOAC Official Methods study (2000.14), the Reveal for *E. coli* O157:H7 20-hour test system was found to be an effective procedure for the detection of *E. coli* O157:H7 in raw ground beef, raw beef cubes, apple cider, iceberg lettuce rinse, and stainless steel environmental swabs.

Assay Principles

This system can utilize various enrichment media to provide *E. coli* O157:H7 with readily available nutrients and other factors required for its survival and rapid growth. Used with Reveal media, the system can provide results within 8–20 hours for 25 g samples, or results within 12–20 hours for 375 g samples.

A portion (120 µL) of the enrichment culture is placed into the round sample port of the Reveal test device. This sample is wicked through a reagent zone that contains specific antibodies (anti-*E. coli* O157:H7) conjugated to colloidal gold particles. If target antigens are present in the sample, they will bind to the gold-conjugated antibodies. This antigen-antibody complex then leaves the reagent zone and travels through the nitrocellulose membrane that contains a zone of anti-*E. coli* O157:H7 antibody. The immune complex with gold conjugate is captured and aggregates in this zone, thus displaying a visible line. The remainder of the sample continues to migrate to the end of the membrane where it will eventually become deposited into a waste reservoir.

The reagent zone also contains gold conjugate of a proprietary antigen (color indicator), which is eluted by the sample solution regardless of the presence of *E. coli* O157:H7 antigen. The gold-conjugated control indicator migrates through the membrane to the negative control capture zone (antibody to the proprietary antigen), where it is captured and aggregated to form a visible line. Regardless of the presence or absence of the *E. coli* O157:H7 antigen, the control line will form in the control zone, ensuring the test is working properly.

Intended User

This test system is designed for use by personnel familiar with the appropriate aseptic techniques for the isolation and identification of *E. coli* O157:H7. Training, which is available through Neogen®, is recommended for those without a basic knowledge of microbiology.

Materials Provided

Reveal Test Kit

1. 20 Reveal for *E. coli* O157:H7 test devices
2. 20 transfer pipettes

Materials Required but Not Provided

1. Stomacher-type bags (Neogen item 9736)
2. Sample bags, 3500 mL (Neogen item 9738)
3. Scale (Neogen item 9427)
4. Timer (Neogen item 9426)
5. Sterile water

For 8-hour Test System:

1. 13 x 100 mm polypropylene or glass tubes (Neogen item 9438)
2. Incubator capable of maintaining 42 ± 1°C (Neogen item 9735)
3. Disposable sterile pipettes capable of transferring 5 mL samples (Neogen item 9415)
4. Water bath or equivalent heating source (e.g., Bunsen burner, heater block (Neogen item 9412), microwave oven)

For 20-hour Test System:

Incubator capable of maintaining 36 ± 1°C (Neogen item 9735)

Optional Materials

Stomacher 400 machine or equivalent

Storage

Store Reveal devices between 2–30°C when not in use. Store dry media at room temperature (15–30°C).

Testing Different Commodities

Reveal for *E. coli* O157:H7 is an effective test for a variety of commodities. Please contact a Neogen representative for an updated list of the validated commodities or a validation study for a specific commodity.

Precautions

1. Do not use expired media.
2. Allow test devices to warm to room temperature before opening pouch.
3. Use rehydrated medium within 6 hours of preparation.
4. Use of incubation times or temperatures other than those specified may cause erroneous results.
5. Sample bags must be closed loosely to allow air exchange during incubation, which is vital to survival and antigenic expression by *E. coli* O157:H7.
6. Sterile water should be brought to 42 ± 1°C before use when using 8 hour medium.
7. Test cultures within 4 hours post incubation if using the 8-hour test system, and within 8 hours post-incubation if using the 20-hour test system.
8. Good microbiological laboratory practices should be used where appropriate.

Sample Preparation and Cultivation

8–hour Test Tystem

1a. 25 g samples

a. Media preparation. Transfer the contents of 1 pouch of unitized Reveal 8 Hour Medium (Neogen item 9760) or 8.9 g of bulk Reveal 8 Hour Medium (Neogen item 9765) into a Stomacher-type bag. Add 225 mL of 42°C sterilized water. Close the bag tightly about 2–3 inches from the top, support bottom, and shake vigorously until medium is dissolved. Place Stomacher-type bag in a rack or other support. The medium can be held at 42°C until used, but no more than 6 hours. For best results, use media soon after it is prepared.

b. Adding sample. Combine 25 g of sample with the media, grasp bag tightly at top and knead until mixed. Shake bag vigorously using a side-to-side motion to ensure complete mixing.
Alternative: Place bag in Stomacher machine and mix for 2 minutes at normal speed.

1b. 375 g samples
   a. Media preparation. Add 133.5 g of Reveal 8 Hour Medium (Neogen items 9765 or 9765B) into a 3500 mL sample bag (Neogen item 9738) and add 3375 mL of 42°C sterile water. Fold the bag over several times 2 or 3 inches from the top, and mix vigorously until dissolved. Place the sample bag in a 2 gallon bucket or other support. The medium can be held at 42°C until used. For best results, use medium soon after it is prepared.
   b Adding sample. Combine 375 g of sample with the media, grasp bag tightly at top and knead until mixed. Shake bag vigorously using a side-to-side motion to ensure complete mixing.
2. Incubation. Close bag loosely. Incubate 25 g samples at 42 ± 1°C for 8 hours. Incubate 375 g samples at 42 ± 1°C for 12 hours. Do not close bag tightly, as air exchange is required for optimum growth and antigen expression.
3. Mixing. Remove the bag from the incubator after 8 hours or 12 hours depending on sample size. Grab the bag tightly 2–3 inches from the top, support bottom, and gently mix the contents of the sample bag using a side-to-side motion.
4. Sample treatment.
   a. Using a sterile pipette, transfer about 5 mL of the sample to a polypropylene or glass tube. Heat sample by:
      • Placing tube in boiling water for 20 minutes if using a polypropylene tube, or 10 minutes if using a glass tube. or
      • Securely capping the tube, placing it into a microwave, bringing to a boil, and quickly turning off the power.
   b. Allow the tube to cool to room temperature (15–30°C). To cool tube faster, place under cool running water. Take care not to let any water get into the tube.

The sample is ready for testing. Proceed to Reveal device test procedure.

20-hour Test System

1a. 25 g sample
   a. Media preparation. Transfer the contents of 1 pouch of unitized Reveal 20 Hour Medium (Neogen item 9770) or 8.3 g of bulk Reveal 20 Hour Medium (Neogen item 9758) into a Stomacher-type bag. Add 225 mL of 36 ± 1°C sterilized water. Close the bag tightly about 2–3 inches from the top, support bottom, and mix vigorously until medium is dissolved (about 1 minute). Place Stomacher-type bag in a rack or other support. The media can be held at 36 ± 1°C until used, but no more than 6 hours. For best results, use media soon after it is prepared.
   b. Adding sample. Combine 25 g of sample with the media, grasp bag tightly at the top and knead until mixed. Shake bag vigorously using a side-to-side motion to ensure complete mixing.
   Alternative: Place in a Stomacher machine and mix for 2 minutes at normal speed.

1b. 375 g samples
   a. Media preparation. Add 124.5 g of Reveal 20 Hour Medium (Neogen items 9758 or 9758B) into a 3500 mL sample bag (Neogen item 9738) and add 3375 mL of 36°C sterile water. Fold the bag over several times, 2–3 inches from the top, and mix vigorously until dissolved. Place the sample bag in a 2 gallon bucket or other support. The medium can be held at 36 ± 1°C until used, but no more than 6 hours. For best results, use media soon after it is prepared.
   b. Adding sample. Combine 375 g of sample with the medium, grasp bag tightly at the top and knead until mixed. Shake bag vigorously using a side-to-side motion to ensure complete mixing.
2. Incubation. Close bag loosely. Incubate sample bag at 36 ± 1°C for 20 hours. Do not close bag tightly, as air exchange is required for optimum growth.
3. Mixing. Remove bag from incubator after 20 hours. Grab the bag tightly 2–3 inches from the top, support the bottom, and gently mix the contents of the sample bag using a side-to-side motion.

Sample types other than ground beef, beef cubes, apple cider, lettuce rinse, and environmental swabs: Follow above instructions. For samples containing high levels of background microflora, increase incubation temperature to 42 ± 1°C.

Alternative enrichments: Alternative 20-hour enrichments for the recovery of *E. coli* O157:H7 include Tryptic Soy Broth, Modified with Novobiocin (Neogen item 7694); Tryptic Soy Broth, Modified with Novobiocin and Acid Digest of Casein (Neogen item 7731); and EC Medium, Modified with Novobiocin (Neogen item 7700). All three enrichments have been or are currently being used by the USDA/FSIS and are available as dehydrated culture media from Neogen as part of the Neogen Culture Media product line.[4,5,6,7]

Sample is ready for testing. Proceed to Reveal device test procedure.

Reveal Device Test Procedure

1. Allow devices to come to room temperature (if stored refrigerated, about 25°C) prior to use.
2. Remove the required number of Reveal for *E. coli* O157:H7 test devices from the foil pouches. Place device on flat surface and label with appropriate sample identification.
3. Squeeze bulb on transfer pipette and insert stem into sample. Release pressure on bulb and draw up sample into stem portion only.
   Caution: For boiled 8-hour samples:
   - Do not disturb sample by vortex, stirring or shaking.
   - Do not disturb particle matter at base of the sample.
   - Remove only the liquid, not debris, from the sample.
4. Hold transfer pipette vertically over the sample port on the device and deliver 5 free falling drops into the port when using either 8 or 20-hour test system.
   Alternative: Using a pipettor, add 120 µL of the sample liquid into the port.
5. Observe and record test results immediately (within 60 seconds) after 15 minutes.

Procedural Note: If Device Flow Problem Exists

If there is no flow in the device after adding the appropriate number of drops of sample to the device, add one more drop. Particulate matter may displace some of the needed volume to initiate the chromatographic flow of the test.

Interpretation of Results

Visual interpretation	Results
Line in C and T zones in 15 minutes	Positive
Line in C zone but no line in T zone	Negative
If no line appears in the C zone, regardless if there is a line in the T zone	Invalid

Confirmation (Optional)

Neogen recommends that presumptively positive enrichment cultures are verified by plating a sample of the enrichment culture onto media described in BAM1 or FSIS procedures.[4, 5, 6, 7]

Note: Testing a replicate subsample using BAM or FSIS procedures may not yield the same result as Reveal. Bacteria may not be evenly distributed within a sample (especially in dry or solid samples), hence the replicate subsample may not contain the organism at the onset.

Disposal

Dispose (autoclave, bleach, etc.) of Reveal test devices, pipettes, and media in accordance with all applicable local, state, and federal regulations.

Customer Service

Neogen Customer and Technical Services can be contacted through Neogen.com and product training is available by request.

SDS Information Available

Safety data sheets are available for all test kits at Neogen.com or by calling 800.234.5333 or 517.372.9200.

Terms and Conditions

Neogen's full terms and conditions are available online.

Warranty

Neogen makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement of the product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product, or of the fitness of the product for anAy purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

References

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[2]Edwards, P.R., and Ewing, W.H., Identification or Enterobacteriaceae, 3rd Ed., Burgess Publishing Co., Minneapolis, MN (1972).

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[5]Cray Jr., W., et al.; Revision #1; 9-6-99 of Laboratory Communication, Chapter 5: Isolation and Identification of *Escherichia coli* O157:H7 from Meat; FSIS publication (1999).

[6]USDA/FSIS Microbiology Laboratory Guidebook, 3rd Ed., Rev. 3 (10/25/02), www.fsis.gov/OPHS/microlab/mlg5.03.pdf.

[7]FSIS Constituent Update, Protecting Public Health Through Food Safety and Food Defense, Volume 9, Issue 3 (01/25/08).

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