



**RapidChek® *E. coli* O157
(including H7) Test Kit**

**Part #: 10001179, 10001355
10001686, 10001687
10001688, 10001356
10001359, 10001360**

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RapidChek® *E. coli* O157 (including H7) Test Kit



AOAC Approved Protocols

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.



Intended Use

The RapidChek® *E. coli* O157 Lateral Flow Test Kit was validated to detect *E. coli* O157 (including H7) in raw ground beef and raw beef trim (AOAC license number 070801). The test kit permits the presumptive detection and identification of the target pathogen in 8, 10 or 12 hours, depending on sample size and matrix tested. The intended user is a laboratory worker with appropriate microbiological training in aseptic technique and the handling of pathogenic bacteria.

Principle of the Test

This immunoassay test uses a double antibody sandwich format. An antibody specific for *E. coli* O157 is sprayed and immobilized in a line on the surface of a membrane comprising a "test line". A second antibody reagent, also recognizing *E. coli* O157 and labeled with colloidal gold, is contained within a reagent pad upstream from the test line. As the sample moves by capillary action from the filter pad, the antibody gold reagent specifically binds *E. coli* O157 and moves with the liquid sample into the test membrane. The sample passes through the test line where the immobilized second *E. coli* antibody captures the protein-antibody-gold complex, causing the formation of an antibody-protein "sandwich" and development of red color at the test line. Antibody-protein sandwiches are not formed in the absence of the *E. coli*, resulting in no red color development at the test line. Reagents immobilized at the control line capture excess gold reagent passing through the test line. The presence of red color at the control line indicates that the test strip flowed correctly. Therefore, the presence of only one line (control line) on the membrane indicates a negative sample and the presence of two lines indicates a positive sample.



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Contents of Kit

10001355

<u>Description</u>	<u>Quantity</u>
RapidChek® <i>E. coli</i> O157 Test Strips (10001179)	50
Transfer Pipettes (1 mL)	50
Test Tubes	50
Package Insert	1

10001686

<u>Description</u>	<u>Quantity</u>
RapidChek® <i>E. coli</i> O157 Test Strips (10001179)	50
Transfer Pipettes (1 mL)	50
Test Tubes	50
Pre-weighed media pouches	10
Package Insert	1

10001687 (or 10001688) (Test Strips)

<u>Description</u>	<u>Quantity</u>
RapidChek® <i>E. coli</i> O157 Test Strips (10001179)	5
Transfer Pipettes (1 mL)	5
Polypropylene Test Tubes	5
RapidChek® <i>E. coli</i> O157 Media	30.2 g (or 500 g)
Package Insert	

10001356

<u>Description</u>	<u>Quantity</u>
RapidChek® <i>E. coli</i> O157 Test Strips (10001179)	500

10001359

<u>Description</u>	<u>Quantity</u>
RapidChek® <i>E. coli</i> O157 Media	500 g

10001360

<u>Description</u>	<u>Quantity</u>
RapidChek® <i>E. coli</i> O157 Media	5 kg



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Storage of Reagents

The RapidChek® *E. coli* O157 Test Kit should be stored at room temperature (15 – 30 °C). The RapidChek® *E. coli* test strips used in this kit must be kept in the canister with the humidity indicating card. The humidity indicating card should be blue in color. After opening the canister, care should be taken to re-close after use to protect the test strips from moisture.

Materials Required but Not Supplied

Stomacher bags or equivalent
Stomacher machine (optional)
Balance with an accuracy of ± 0.2 g
Incubator capable of maintaining 42 ± 1 °C

For the RapidChek® Procedure:
RapidChek® *E. coli* Media (P/N 10001359)

For the mTSB Procedure:
Modified Trypticase Soy Broth (Oxoid Product #CM0989B or equivalent) with novobiocin plus casaminoacids (casein acid hydrolysate).



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Media Preparation and Sample Enrichment For RapidChek® Media System

A. Media Preparation

1. Weigh 25.2 ± 0.2 g of RapidChek® *E. coli* O157 Media and add to 1 liter of deionized water. Shake vigorously until the media is dissolved.
2. Autoclave for 15 minutes at 121 °C or filter sterilize (pore size of 0.2 μm) the rehydrated media.

Note: Autoclaved media may be stored for up to 4 weeks at $2 - 8$ °C or room temperature. Filter sterilized media may be stored for up to 2 weeks at $2 - 8$ °C.

3. Alternatively, rehydrate media in one liter of sterile, deionized water equilibrated to 42 ± 1 °C. In this manner, rehydrated media should be used within 3 hours of preparation. For best results, use the media as soon as it is prepared.

B. Sample Enrichment: 25 g Raw Beef Sample

1. Add 225 mL RapidChek media (pre-warmed to 42 ± 1 °C) to a sterile Stomacher-type bag.
2. Add 25 g of the sample to be analyzed into the Stomacher bag containing the media.
3. Place the sample bag into a Stomacher device and stomach for 45 seconds or hand massage the bottom of the bag.
4. Close the bag loosely and incubate for 8 hours at 42 ± 1 °C.
5. After 8 hours, remove the bag from the incubator and gently mix contents using a gentle swirling motion.
6. Proceed to the RapidChek® *E. coli* O157 detection procedure.



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C. Sample Enrichment: 375 g Raw Ground Beef Sample

1. Add 3.375 L RapidChek® media (pre-warmed to 42 ± 1 °C) to a sterile Stomacher-type bag.
2. Add 375 g of the ground beef sample to be analyzed into the Stomacher bag containing the media.
3. Place the sample bag into a Stomacher device and stomach for 45 seconds or hand massage the bottom of the bag.
4. Close the bag loosely and incubate for 12 hours at 42 ± 1 °C. Samples may be incubated up to 18 hours without compromising the accuracy of the test.
5. After 12 hours, remove the bag from the incubator and gently mix contents using a gentle swirling motion.
6. Proceed to the RapidChek® *E. coli* O157 detection procedure.

D. Sample Enrichment: 375 g Raw Beef Trim Sample

1. Add 1.5 L RapidChek® media (pre-warmed to 42 ± 1 °C) to a sterile Stomacher-type bag.
2. Add 375 g of the beef trim sample to be analyzed into the Stomacher bag containing the media.
3. Place the sample bag into a Stomacher device and stomach for 45 seconds or hand massage the bottom of the bag.
4. Close the bag loosely and incubate for 10 hours at 42 ± 1 °C. Samples may be incubated up to 18 hours without compromising the accuracy of the test.
5. After 10 hours, remove the bag from the incubator and gently mix contents using a gentle swirling motion.
6. Proceed to the RapidChek® *E. coli* O157 detection procedure.



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E. Optional: Heat-Killed Organisms

1. After removing the enrichment from the incubator, transfer 5 mL of sample to a glass tube using a sterile pipette.
2. Heat sample by placing tube in boiling water bath or heat block for 10 minutes. Allow the tube to cool to room temperature.
3. Proceed to the RapidChek® *E. coli* O157 detection procedure.

Alternative Method: Media Preparation and Sample Enrichment For mTSB with Novobiocin plus Casaminoacids

A. Media Preparation

1. Weigh 33 g of mTSB (Oxoid Product #CM0989B or equivalent) and 10 g of casaminoacids (casein acid hydrolysate) per liter of distilled water.
2. Shake vigorously until the media is dissolved.
3. Autoclave for 20 minutes at 121 °C.
4. Let the media cool and add 20 mg/L of novobiocin.
5. Equilibrate the media to 42 ± 1 °C.

B. Sample Enrichment: 375 g Raw Ground Beef Sample

1. Add 3.375 L mTSB broth (pre-warmed to 42 ± 1 °C) to a sterile Stomacher-type bag.
2. Add 375 g of the ground beef sample to be analyzed into the Stomacher bag containing the media.
3. Place the sample bag into a Stomacher device and stomach for 45 seconds or hand massage the bottom of the bag.
4. Close the bag loosely and incubate for 15 hours at 42 ± 1 °C.



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5. After 15 hours, remove the bag from the incubator and gently mix contents using a gentle swirling motion.
6. Proceed to the RapidChek® *E. coli* O157 detection procedure.

C. Sample Enrichment: 375 g Raw Beef Trim Sample

1. Add 3.375 L mTSB broth (pre-warmed to $42 \pm 1^\circ\text{C}$) to a sterile Stomacher-type bag.
2. Add 375 g of the beef trim sample to be analyzed into the Stomacher bag containing the media.
3. Place the sample bag into a Stomacher device and stomach for 45 seconds or hand massage the bottom of the bag.
4. Close the bag loosely and incubate for 15 hours at $42 \pm 1^\circ\text{C}$.
5. After 15 hours, remove the bag from the incubator and gently mix contents using a gentle swirling motion.
6. Proceed to the RapidChek® *E. coli* O157 detection procedure.

D Optional: Heat-Killed Organisms

1. After removing the enrichment from the incubator, transfer 5 mL of sample to a glass tube using a sterile pipette.
2. Heat sample by placing tube in boiling water bath or heat block for 10 minutes. Allow the tube to cool to room temperature. Proceed to the RapidChek® *E. coli* O157.



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RapidChek® *E. coli* O157 Detection Procedure

1. Take one transfer pipette from the bag (or utilize a calibrated pipette capable of dispensing 1 mL). Squeeze and hold the top of the pipette and place in the sample enrichment.
2. Release the bulb filling the barrel of the pipette up to the 1 mL mark.
Note: Sample volumes greater than 1 mL can produce an inaccurate result.
3. Transfer the aliquot of enriched broth to the supplied plastic tubes.
4. Remove the required number of test strips from the canister.
5. Insert the strip with arrows facing down into the tube.
6. Let the strip develop for 10 minutes.
7. The appearance of one red line (control) on the strip indicates a negative result.

Verification of a RapidChek® *E. coli* O157 Potential Positive as a Presumptive *E. coli* O157:H7 Result

After a RapidChek® strip positive result, an additional PCR screening test can be performed directly on the RapidChek® enrichment to determine the presence of *E. coli* O157:H7 without the need for further enrichment. Follow the manufacturer's instructions to perform the PCR test. Proceed to confirmation procedure following a presumptive positive result.

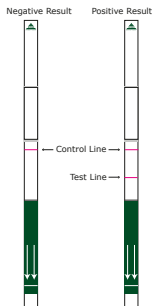
Note: Please contact Romer Labs' Technical Service at 855-337-6637 for guidance on the appropriate PCR test for your application.



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Illustration of Positive and Negative Results



At least one line, the Control Line, should always develop. A red line in this position indicates that the strip is functioning properly. If the test strip displays 2 red lines, the test is complete and the sample is positive for *E. coli* O157 Including H7.

If at 10 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for *E. coli* O157 Including H7. If no control line develops within 10 minutes, the test is invalid and needs to be repeated.

Note: Test strip results should be interpreted after 10 minutes.

Test strips interpreted after 20 minutes are invalid.

Confirmation

Potential (and presumptive) positive samples should be confirmed according to the USDA/FSIS Microbiology Laboratory Guidebook (Chapter 5.06) for the detection of *E. coli* O157:H7 in meat products. Enriched media samples used in the RapidChek® *E. coli* O157 Test Procedure can be used for this confirmation.

Disposal

Decontaminate RapidChek® test strips, pipettes and media by autoclave, bleach, etc., in accordance with local, state and federal regulations.

Product Shelf life

The expiration date for the product is displayed along with the part and lot number on the Product Label located on the canister. This product has a one year shelf life from the date of manufacture under desiccated room temperature conditions. Contact customer service with any questions about product shelf life.



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Precautions

1. *E. coli* O157:H7 has a very low infective dose (approximately 50 cells). Extreme care should be used in handling samples, enriched media and used test strips.
2. If polypropylene bottles are used for sample enrichment instead of Stomacher bags, the bottles should be lined with a disposable plastic bag to eliminate potential protein carryover, which will produce erroneous results.
3. Storage conditions higher than room temperature may adversely affect performance of the test strip.
4. Follow standard Good Microbiological Practices where appropriate to prevent contamination of samples, lab workers and the environment.

Warranty and Liabilities

The user assumes all risk in using Romer Labs products and services. Romer Labs will warrant that its products and services meet all quality control standards set by Romer Labs and Romer Labs will, at its option, repair or replace any product, components, or repeat services which prove to be defective in workmanship or material within product specific warranty periods or expiration dates and which our examination shall disclose to our satisfaction to be defective as such. This warranty is expressly in lieu of all other warranties, expressed or implied, as to description, quality, merchantability, fitness for any particular purpose, productiveness, or any other matter. Romer Labs shall be in no way responsible for the proper use of its products. Romer Labs, Inc hereby disclaims all other remedies, warranties, guarantees or liabilities, expressed or implied, arising by law or otherwise, and it shall have no liability for any lost profits or damage, direct, indirect or otherwise, to person or property, in connection with the use of any of its products or services. This warranty shall not be extended, altered or varied except by a written instrument signed by an authorized representative of Romer Labs.



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