MicroSEQ® *E. coli* 0157:H7 Detection Kit

**Catalog Numbers** 4427409, 4445654 (with protocol and quick reference card), 4445656 (with PrepSEQ® Nucleic Acid Extraction Kit), 4445657 (with PrepSEQ® Rapid Spin Sample Prep Kit)

**Publication Number** 4426511

**Revision** C

**PERFORMANCE TESTED**

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**IMPORTANT!** Before using this product, read and understand the information in Appendix C, “Safety” on page 45.

**WARNING!** *E. coli* O157:H7 is a Biosafety Level 2 (BSL-2) organism. Care must be taken when handling samples that may contain *E. coli* O157:H7. Laboratory personnel must be adequately trained to handle pathogens before being permitted to analyze samples for *E. coli* O157:H7. Laboratory personnel must wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. Extreme precautions should be taken with contaminated sharp items. Access to the laboratory should be limited when work is being conducted. Waste should be disposed of in compliance with local and national legislation as appropriate.

Ensure that your instrument is properly installed and calibrated. For calibration information, see the documentation that is provided with your instrument.

### Product description

The MicroSEQ® *E. coli* O157:H7 Detection Kit detects *E. coli* O157:H7 simply, reliably, and rapidly in food samples using a lyophilized reagent format. The assay uses the polymerase chain reaction (PCR) to amplify unique microorganism-specific DNA target sequences and TaqMan® probes to detect the amplified sequences.

You can use the RapidFinder™ Express software, Sequence Detection System (SDS) software, or StepOne® software with this detection kit to prepare for and manage the detection process. This protocol provides instructions for:

- Automating detection using the RapidFinder™ Express software (**Chapter 1**)
- Performing detection using the SDS software (**Chapter 2**)
- Performing detection using the StepOne® software (**Chapter 3**)

For additional background information, refer to Appendix A on page 39.
# Materials and equipment

## Kit contents

MicroSEQ® *E. coli* O157:H7 Detection Kit contain reagents for 96 reactions. Kit components and their storage conditions are shown in the following table.

<table>
<thead>
<tr>
<th>Cat. no.</th>
<th>Contents</th>
<th>Cap color</th>
<th>Description</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>4427409</td>
<td>MicroSEQ® <em>E. coli</em> O157:H7 Detection Kit (Part 1 of 2)</td>
<td>Orange (rack)</td>
<td><em>E. coli</em> O157:H7 Assay Beads, twelve 8-tube strips (96 reactions/kit)</td>
<td>5 ±3°C; protect from light‡, seal pouch tightly§</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>MicroAmp® Optical 8-Cap Strips, 1 bag, twelve 8-cap strips</td>
<td>Room temperature (23 ±5°C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red</td>
<td>Pathogen Detection Negative Control, 1 tube, 1.5 mL of control (Cat. no. 4403926)</td>
<td>5 ±3°C</td>
<td></td>
</tr>
<tr>
<td>4445654</td>
<td>MicroSEQ® <em>E. coli</em> O157:H7 Detection Kit</td>
<td></td>
<td></td>
<td>For storage conditions of MicroSEQ® components, see above.</td>
</tr>
<tr>
<td></td>
<td>• Contents of Cat. no. 4427409 (see above)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• User Guide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Quick Reference Card</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4445656</td>
<td>MicroSEQ® <em>E. coli</em> O157:H7 Detection Starter Kit with PrepSEQ® Nucleic Acid Extraction Kit</td>
<td></td>
<td></td>
<td>For storage conditions of MicroSEQ® components, see above.</td>
</tr>
<tr>
<td></td>
<td>• Contents of Cat. no. 4427409 (see above)</td>
<td></td>
<td></td>
<td>PrepSEQ® components:</td>
</tr>
<tr>
<td></td>
<td>• PrepSEQ® Nucleic Acid Extraction Kit (Magnetic Particles, Lysis Buffer, Wash Buffer Concentrate, Elution Buffer, Proteinase K Buffer, Proteinase K)</td>
<td></td>
<td></td>
<td>• Magnetic Particles: 5 ±3°C</td>
</tr>
<tr>
<td></td>
<td>• User Guides</td>
<td></td>
<td></td>
<td>• Buffers: 23 ±5°C</td>
</tr>
<tr>
<td></td>
<td>• Quick Reference Cards</td>
<td></td>
<td></td>
<td>• Proteinase K: Below –18°C</td>
</tr>
<tr>
<td>4445657</td>
<td>MicroSEQ® <em>E. coli</em> O157:H7 Detection Starter Kit with PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K</td>
<td></td>
<td></td>
<td>For storage conditions of MicroSEQ® components, see above.</td>
</tr>
<tr>
<td></td>
<td>• Contents of Cat. no. 4427409 (see above)</td>
<td></td>
<td></td>
<td>PrepSEQ® Kit:</td>
</tr>
<tr>
<td></td>
<td>• PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K (Lysis Buffer, 100 Spin Columns, 200 Collection tubes, Proteinase K)</td>
<td></td>
<td></td>
<td>• Lysis Buffer: 5 ±3°C</td>
</tr>
<tr>
<td></td>
<td>• User Guides</td>
<td></td>
<td></td>
<td>• Columns &amp; Tubes: 23 ±5°C</td>
</tr>
<tr>
<td></td>
<td>• Quick Reference Cards</td>
<td></td>
<td></td>
<td>• Proteinase K: Below –18°C</td>
</tr>
</tbody>
</table>

‡ Excessive exposure to light may affect the fluorescent probes.
§ To protect assay beads from moisture, seal the pouch tightly each time you remove an 8-tube strip from the pouch.

**Note:** Parts may ship separately depending on configuration and storage conditions.
The following table includes materials that are required for using but not included in the MicroSEQ® *E. coli* O157:H7 Detection Kit (Cat. no. 4427409). Unless otherwise indicated, many of the listed items are available from major laboratory suppliers (MLS).

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Instruments, equipment, consumables, and reagents‡</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Instruments</strong></td>
<td></td>
</tr>
<tr>
<td>Applied Biosystems® 7500 Fast Real-Time PCR System</td>
<td>Contact your local Life Technologies sales office.</td>
</tr>
<tr>
<td>StepOnePlus® Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td><strong>Equipment</strong></td>
<td></td>
</tr>
<tr>
<td>Benchtop microcentrifuge</td>
<td>MLS (Major laboratory suppliers)</td>
</tr>
<tr>
<td>Plate centrifuge</td>
<td>MLS</td>
</tr>
<tr>
<td>Vortexer</td>
<td>MLS</td>
</tr>
<tr>
<td>MicroAmp® 96-Well Base (optional)</td>
<td>Life Technologies Cat. no. N8010531</td>
</tr>
<tr>
<td>MicroAmp® Cap Installing Tool</td>
<td>Life Technologies Cat. no. 4330015</td>
</tr>
<tr>
<td>7500 Fast Precision Plate Holder for MicroAmp® Tube Strips§ (for use with 7500 Fast Real-Time PCR System)</td>
<td>Life Technologies Cat. no. 4403809</td>
</tr>
<tr>
<td>MicroAmp® 96-Well Tray for Veriflex™ Blocks (for use with StepOnePlus® Real-Time PCR System)</td>
<td>Life Technologies Cat. no. 4379983</td>
</tr>
<tr>
<td><strong>Consumables</strong></td>
<td></td>
</tr>
<tr>
<td>PrepSEQ® Nucleic Acid Extraction Kit – Food and Environmental Testing# (includes optional Proteinase K)</td>
<td>Life Technologies Cat. no. 4428176</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit</td>
<td>Life Technologies Cat. no. 4407760</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit with Proteinase K</td>
<td>Life Technologies Cat. no. 4426714</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean</td>
<td>Life Technologies Cat. no. 4413269</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K‡‡</td>
<td>Life Technologies Cat. no. 4426715</td>
</tr>
</tbody>
</table>
### Instruments, equipment, consumables, and reagents

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerosol-resistant pipette tips</td>
<td>MLS</td>
</tr>
<tr>
<td>Disposable gloves</td>
<td>MLS</td>
</tr>
<tr>
<td>Pipettors:</td>
<td>MLS</td>
</tr>
<tr>
<td>• Positive-displacement</td>
<td></td>
</tr>
<tr>
<td>• Air-displacement</td>
<td></td>
</tr>
<tr>
<td>• Multichannel</td>
<td></td>
</tr>
<tr>
<td>MicroAmp® Fast 8-Tube Strip, 0.1-mL</td>
<td>Life Technologies Cat. no. 4358293</td>
</tr>
<tr>
<td>MicroAmp® Optical 8-Cap Strip, 300 strips</td>
<td>Life Technologies Cat. no. 4323032</td>
</tr>
</tbody>
</table>

### Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease-free Water</td>
<td>Life Technologies Cat. no. AM9938</td>
</tr>
</tbody>
</table>

‡ The materials listed here have been validated for use with this kit. Results may vary if substituted products from other vendors are used instead.
§ Included in the starter kit.
# Included with Cat. no. 4445656
‡‡ Included with Cat. no. 4445657
Kit workflow

This chapter presents a protocol using the RapidFinder™ Express software on the 7500 Fast instrument to automate MicroSEQ® E. coli O157:H7 Detection Kit processes.

1. **Isolate DNA** (page 10)
   - Pre-enrichment
   - Sample preparation

2. **Prepare for PCR** (page 10)
   - Create or edit a run file using the RapidFinder™ Express software
     - Enter run file information
     - Select targets
     - Specify samples and controls
     - Enter sample names
     - Review and confirm the run layout

3. **Pipet samples**
   - Select a run file
   - Select a pipetting procedure option
   - Set up sample and control tubes
   - Pipet unknowns, negative controls, and positive controls
   - Load the instrument

4. **Start instrument run** (page 15)
   - Select a file to run
   - Perform the run
   - Unload the instrument

5. **View results** (page 15)
   - View results by location and by sample
   - Print reports
   - Export results
Isolate DNA

One of the following methods of isolating DNA is recommended with this detection kit. The following two documents describing these methods contain information on pre-enrichment and sample preparation using PrepSEQ® kits:

- **PrepSEQ® Nucleic Acid Extraction Kit User Guide: E. coli O157:H7**
  (automated magnetic bead-based protocol: Pub. no. 4426513)

<table>
<thead>
<tr>
<th>Kit</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrepSEQ® Nucleic Acid Extraction Kit – Food and Environmental Testing (includes optional Proteinase K)</td>
<td>4428176</td>
</tr>
</tbody>
</table>

- **PrepSEQ® Rapid Spin Sample Preparation Kits User Guide: E. coli O157:H7**
  (spin column-based protocol: Pub. no. 4426519)

<table>
<thead>
<tr>
<th>Kit‡</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit</td>
<td>4407760</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit with Proteinase K</td>
<td>4426714</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean</td>
<td>4413269</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K</td>
<td>4426715</td>
</tr>
</tbody>
</table>

‡ We recommend extra clean for matrices containing high fat content. Most food samples derived from animal sources show improved recovery of E. coli DNA with the addition of Proteinase K digestion, but no improvement was observed with non-animal products.

Prepare for PCR

To prepare for PCR, first open RapidFinder™ Express software from the 7500 Fast instrument and create a run file document, then prepare the assay beads and samples. We recommend using the RapidFinder™ Express software for online step-by-step instructions to set up the assays followed by automated data analysis. For details, refer to the RapidFinder™ Express Software Online Help (Pub. no. 4401842).
Start the RapidFinder™ Express software

1. Click the RapidFinder™ Express icon to access the Main page:

![RapidFinder™ Express Software](image)

**IMPORTANT!** Ensure that your RapidFinder™ Express software version is a version that includes the target pathogen *E. coli* O157:H7.

Create or edit a run file using RapidFinder™ Express Software

1. On the Main page of the RapidFinder™ Express software, click **Create/Edit a Run File**.

2. Select **Create New Run File**, **Edit Existing Run File**, or **Use Run File Template**. Click **Next** to continue.

3. Enter or edit the Run File information. Click **Next** to continue.

   **Note:** A Run File Name is required in order to continue.

4. Select the target pathogen to test, for example *E. coli* O157:H7, then enter the number of samples, replicates, and positive and negative controls for each target. At least one negative control is required for each target pathogen. Click **Next** to continue.

   **Note:** Different target pathogens assays can be run at the same time.
5. Enter or import samples names and information for each target. Click **Next** to continue.

**Note:** Sample names can be imported if an import file was created.

6. Review and confirm the Run Layout. Click **Print Run Layout** if you would like a printout of the page. If the run file is set up correctly, click **Next** to continue.

7. Select **Pipette Samples** to directly proceed with viewing pipetting instructions, and click **Next** to continue. Or click **Close** to return to the Main Page.

---

### Pipet samples

1. On the Main page of the RapidFinder™ Express software, click **Pipette Samples**. Select a run file for pipetting, then click **Next**. (If you selected Pipette Samples on the last page of Create a Run File, the run file you just created is selected by default.)

2. Select **Print all pipetting procedures, Print only the run layout, Display the run layout, or Use online step-by-step pipetting procedures**. Proceed with your pipetting procedure selection as prompted by the software.

3. Open the storage pouch containing the assay beads (cut at the notch in the upper corner of the storage pouch above the zip-lock strip).

**IMPORTANT!** Do not remove the desiccant from the storage pouch.

4. Remove the appropriate number of individual tubes or 8-tube strips and place in a 96-well base, as directed by the software in the Run Layout or the Pipette Samples instructions. Remove the colored caps and discard. Avoid disturbing the beads from the bottom of the tubes.

**Note:** Assay beads contain all the components necessary for each reaction.

**IMPORTANT!** Do not use colored caps or tubes for kit reaction. Colored caps or tubes may affect dye-signal readings during real-time PCR.

**Note:** The RapidFinder™ Express software layout is displayed using the abbreviations L. mono for *Listeria monocytogenes*, Sal spp. for *Salmonella* species, O157:H7 for *Escherichia coli* O157:H7, and L. species for *Listeria* species.

The Run Layout uses icons to indicate unknown sample, negative control, positive control, or an empty tube in each position.

---

MicroSEQ® E. coli O157:H7 Detection Kit User Guide
5. Seal the storage pouch using the zip-lock strip, then store at 5 ±3°C.

6. Follow the directions of the online step-by-step pipetting procedures (or print instructions) to pipet unknown samples, negative controls, and positive controls. Add 30 µL of sample to each assay bead using the same configuration as the run layout determined by the RapidFinder™ Express software. Beads dissolve in 1–5 seconds. Mix by gently aspirating and dispensing a few times. (Alternatively, vortex assay tubes after they are capped in the final step.)

**IMPORTANT!** Condensation can occur during storage. Remove condensation after thawing samples and prior to opening to avoid cross contamination.
- For samples prepared using the PrepSEQ® Nucleic Acid Extraction Kit, centrifuge the plate at 1000–2000 × g for approximately 1 minute.
- For samples prepared using the PrepSEQ® Rapid Spin Sample Preparation Kit, microcentrifuge the tubes at 12,000–16,000 × g for approximately 1 minute to bring down any particulate material derived from the spin column, which can interfere with amplification. The microbial DNA is in the aqueous phase.
- For Pathogen Detection Negative Control and positive control(s) in microcentrifuge tubes, vortex the tubes, and then spin down.

**IMPORTANT!** You must follow the layout determined by the RapidFinder™ Express software.

**Note:** Add 30 µL of the PrepSEQ® Nucleic Acid Extraction Kit sample or 30 µL of the PrepSEQ® Rapid Spin Kit sample to each assay bead. The PrepSEQ® Nucleic Acid Extraction Kit provides ~100 µL of sample, and the PrepSEQ® Rapid Spin Kit provides 300 µL of sample. Use 30 µL of the Pathogen Detection Negative Control per negative control reaction. If you use other sample preparation methods, and less than 30 µL of sample is available, adjust the sample volume with water to 30 µL for each reaction type.

**Note:** Unknown samples and positive control samples are provided by the investigator. The kit includes a negative control (Pathogen Detection Negative Control).

7. Arrange empty tubes in the 96-well base as indicated in the layout to balance the tray when the assay tubes are placed in the instrument later.

8. Cap the tubes, sealing each tube with the transparent optical strip caps using a MicroAmp® 96-Well Base (Cat. no. N8010531) and the MicroAmp® Cap Installing Tool (Cat. no. 4330015) to avoid collapsing, bending, or misaligning the tubes. Confirm that the strips are straight and that each tube is in line with the adjacent tube.
9. Mark or label one end of the strip cap (but not directly over any one cap) so that you will remember the orientation when transferring the tubes to the instrument tray.

10. Make sure that the reactions are thoroughly mixed; if reactions were not previously mixed during the pipetting step, place the assay tubes in a 96-well base and use a vortexer to mix.

11. Make sure that the reagents are at the bottom of the tubes; if available, spin down the tube contents at 1000–2000 × g for approximately 30 seconds using a centrifuge with a plate adapter.

12. To load the instrument, transfer the tubes to the 7500 Fast Instrument in the same configuration as the run layout. Use the 7500 Fast Precision Plate Holder for MicroAmp® Tube Strips (Cat. no. 4403809; see the following figure).

13. Close the tray for the instrument (see the following figure).

14. When finished with the pipetting procedure, the software will prompt you for the next step (for example, Start Instrument Run), or click Close to return to the Main page.

<table>
<thead>
<tr>
<th>Press the tray door to open it.</th>
<th>Transfer the tubes to the precision plate holder (PPH) in the tray.</th>
<th>Close the tray door.</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image 1" /></td>
<td><img src="image2.png" alt="Image 2" /></td>
<td><img src="image3.png" alt="Image 3" /></td>
</tr>
</tbody>
</table>
Start instrument run

1. On the Main page, click Start Instrument Run. Select a run file to run, then click Next. (If you selected Start Instrument Run on the last page of Pipette Samples, the run file you just pipetted is selected by default.)

2. Click on the Start Instrument Run page to perform the run.

3. When the run is complete, unload the tubes from the instrument.

View results

1. On the Main page, click View Results. Select a run file to view.

2. Click the Results by Location tab to examine the results for all the tubes. The default Layout View displays results as icons. Click on the Table View radio button to display results and assessment in text format. Click Help for a description of assessment text and suggested actions for warning results.

3. Click the Results by Sample tab to examine results for all replicate samples for a given sample/target combination. Click Help for a description of assessment text and suggested actions for warning results.

Resources for viewing results

For more information about analyzing your data, refer to the:
- Appropriate instrument user guide
- RapidFinder™ Express Software Online Help (Part no. 4401842)

We do not recommend using the same method to screen samples and to confirm results. When you use the MicroSEQ® Pathogen Detection System to screen samples, culture and biochemical methods are recommended to confirm the result. FSIS (USDA) MLG 5.04 and ISO 16654:2001, (see “References” on page 51) are approved protocols for confirming E. coli O157:H7. In the context of NF Validation, positive results must be confirmed as described in Appendix A, “ISO 16140 Validation” on page 40.
Investigate results in the SDS software

As needed, to obtain more information on warning results, investigate results in the SDS software.

1. On the Main page of the RapidFinder™ Express software, select View Results.

2. Select a run file to view.

3. Click View in SDS from within the RapidFinder™ Express software (see figure labeled, “‘View in SDS’ button”) to open SDS software (see figure labeled, “SDS software”). Click on the Results tab. For more information, see “Investigating Warning Results or Failed Runs in the SDS Software” in the RapidFinder™ Express Software Online Help (Part no. 4401842).

**IMPORTANT!** If you modify a RapidFinder™ Express software run file in the SDS software, you cannot open the run file again in the RapidFinder™ Express software. To avoid altering a RapidFinder™ Express software run file, save the run file under a new name in the SDS software before performing any actions.

Print and export results

To print a report in the RapidFinder™ Express software:

1. In the View Results page, click the Report tab.

2. Select the options to include on the report. Click Print.

To export results:

3. In the View Results page, click the Report tab.

4. Select the options to include in the exported results. Click Export Results.
# Kit workflow

This chapter presents a protocol for using the MicroSEQ® *E. coli* O157:H7 Detection Kit with the Sequence Detection System (SDS) software on the 7500 Fast instrument.

### Isolate DNA
- Pre-enrichment
- Sample preparation

### Prepare for PCR
- Create a run file document using SDS software
- Prepare the assay beads
- Prepare samples and controls
- Combine samples and controls with assay beads
- Prepare tubes for the 7500 Fast System

### Start instrument run
- Run the 8-tube reactions on the 7500 Fast System

### View results on the 7500 Fast System

**Note:** The SDS software was not included in either the AOAC or AFNOR validation studies.
Isolate DNA

One of the following methods of isolating DNA is recommended with this detection kit. The following two documents describing these methods contain information on pre-enrichment and sample preparation using PrepSEQ® kits:

- **PrepSEQ® Nucleic Acid Extraction Kit User Guide: E. coli O157:H7** (automated magnetic bead-based protocol: Pub. no. 4426513)

<table>
<thead>
<tr>
<th>Kit</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrepSEQ® Nucleic Acid Extraction Kit – Food and Environmental Testing (includes optional Proteinase K)</td>
<td>4428176</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Kit</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit</td>
<td>4407760</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit with Proteinase K</td>
<td>4426714</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean</td>
<td>4413269</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K</td>
<td>4426715</td>
</tr>
</tbody>
</table>

‡ We recommend extra clean for matrices containing high fat content. Most food samples derived from animal sources show improved recovery of *E. coli* DNA with the addition of Proteinase K digestion, but no improvement was observed with non-animal products.

Prepare for PCR

To prepare for PCR, first open the SDS software from the 7500 Fast instrument and create a run file document, then prepare the assay beads and samples. This chapter describes the basic procedure that uses SDS software for the 7500 Fast Real-Time PCR System.
Start the SDS software

Click the SDS icon to open the SDS software for the 7500 Fast System:

Create a run file document using SDS software

1. In the SDS software, create a run file document. Go to File ➤ New to open a New Document Wizard.

2. In the Define Document section of the New Document Wizard, select the following:
   a. Assay: **Absolute Quantification** (Standard Curve).
   b. Container: **96-Well Clear**.
   c. Template: **Blank Document**.
   d. Run Mode: **Fast 7500**.
   e. For all other fields, enter the requested information.
   f. Click Next to continue.

   **Note:** For more information on creating a run file document, refer to the documentation that is provided with your instrument.

3. In the Select Detectors section of the New Document Wizard, find the detectors that contain the Reporters set at **FAM**, **VIC**, and **NED**, and with Quenchers set at **none**. Select each detector and click Add to enter the detector into the Detectors in Document field. Click Next to continue.

   **Note:** If a detector is missing from the list, then click New Detector and create a new detector for each reporter dye. When creating new detectors it is recommended to select a different color for each reporter dye.
4. In the Set Up Sample plate section of the New Document Wizard, assign the appropriate reporter dyes to each well that contains sample and click on the Use checkbox next to each detector. Click Finish to continue.

**IMPORTANT!** Review “Start instrument run” on page 24 to determine the optimal layout in order to minimize bending or misaligning the tube strips.

**Note:** If FAM™, VIC®, and NED™ detectors are missing from the list of detectors, use the Back tab to add the missing detectors to the document. Each well that contains sample should contain three “U” symbols that correspond to the three reporter dyes, FAM™, VIC®, and NED™ dyes.

5. Under the Setup tab, enter sample names for each sample by double-clicking the sample well.

**Note:** Each well that contains sample should contain three “U” symbols that correspond to the three reporter dyes from FAM™, VIC®, and NED™ dyes. The NED™ dye is used to detect the internal positive control (IPC); the FAM™ and VIC® dyes are used to detect the targets.


<table>
<thead>
<tr>
<th>Step</th>
<th>Enzyme activation</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HOLD</td>
<td>Cycle (40 cycles)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Denature</td>
</tr>
<tr>
<td>Temp.</td>
<td>95°C</td>
<td>95°C</td>
</tr>
<tr>
<td>Time</td>
<td>2 min</td>
<td>3 sec</td>
</tr>
</tbody>
</table>
Chapter 2  
MicroSEQ® E. coli O157:H7 Detection Kit  
Prepare for PCR

7. Set the Sample Volume to 30 µL.  
The following image shows thermal cycling conditions for the 7500 Fast System using SDS software with run mode set to Fast 7500:

![Thermal Cycler Protocol](image)

8. Under File, save the run file as an .sds document in the appropriate folder.

Prepare the assay beads

1. Open the storage pouch containing the assay beads (cut at the notch in the upper corner of the storage pouch above the zip-lock strip).

   **IMPORTANT!** Do not remove the desiccant from the storage pouch.

2. Remove the appropriate number of individual tubes or 8-tube strips based on the number of samples and controls that you plan to run, and place them in a 96-well base. At least one negative control is recommended for the target pathogen. Remove colored caps and discard. Avoid disturbing the beads from the bottom of the tubes.

   **Note:** The assay beads contain all the components necessary for each reaction.

   **IMPORTANT!** Do not use colored caps or tubes for kit reactions. Colored caps or tubes may affect dye-signal readings during real-time PCR.

3. Seal the storage pouch using the zip-lock strip, then store at 5 ±3°C.
Prepare samples and controls

1. Thaw all reagents (samples and controls) completely.

2. Remove condensation after thawing samples and prior to opening to avoid cross contamination.
   - For PrepSEQ® Nucleic Acid Extraction Kit samples, centrifuge the plate at 1000–2000 × g for approximately 1 minute.
   - For PrepSEQ® Rapid Spin Sample Preparation Kit samples, microcentrifuge the tubes at 12,000–16,000 × g for approximately 1 minute to bring down any particulate material derived from the spin column, which can interfere with amplification. The microbial DNA is in the aqueous phase.
   - For Pathogen Detection Negative Control and positive control(s) in microcentrifuge tubes, vortex the tubes, and then spin down.

   **Note:** Unknown samples and positive control samples are provided by the investigator. The kit includes a negative control (Pathogen Detection Negative Control).

3. Add 30 µL of sample prepared above to each assay bead. Dispense all unknown samples first, followed by negative control(s) and then positive control(s). Beads dissolve in 1–5 seconds. Mix by gently aspirating and dispensing a few times. (Alternatively, vortex assay tubes after they are capped in the final step.)

   **IMPORTANT!** Use a new pipette tip for each sample. Resuspend by gently pipetting up and down with the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination or, if a vortexer is available, vortex the capped tubes until the pellet is dissolved. See Appendix B, “Good PCR Practices” on page 43 for more information.

   **Note:** Add 30 µL of the PrepSEQ® Nucleic Acid Extraction Kit sample or 30 µL of the PrepSEQ® Rapid Spin Kit sample to each assay bead. The PrepSEQ® Nucleic Acid Extraction Kit provides ~100 µL of sample, and the PrepSEQ® Rapid Spin Kit provides 300 µL of sample. Use 30 µL of the Pathogen Detection Negative Control per negative control reaction. If you use other sample preparation methods, and less than 30 µL of sample is available, adjust the sample volume with water to 30 µL for each reaction type.
Prepare tubes for the 7500 Fast System

8-tube strips containing assay beads are compatible with the 7500 Fast System.

1. For 8-tube strips with seven or fewer reactions, add additional empty tubes as needed so that each strip contains a full set of 8 tubes.

   **Note:** The empty capped 8-tube strips evenly distribute the clamping load that is applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes. Add empty tubes as needed to create a complete 8-tube strip and cap with an intact 8-cap strip.

2. Cap the tubes, sealing each tube with the transparent optical strip caps provided in the kit. Cap the tubes with the strip caps using the MicroAmp® 96-Well Base (Cat. no. N8010531) and the MicroAmp® Cap Installing Tool (Cat. no. 4330015) to avoid collapsing, bending, or misaligning the tubes. Confirm that the strips are straight and that each tube is in line with the adjacent tube.

3. Mark or label one end of the strip cap (but not over a cap) so that you will remember the orientation when transferring the tubes to the instrument tray.

4. Make sure that the reactions are thoroughly mixed. If reactions were not previously mixed during the pipetting step, place the assay tubes in a 96-well base and use a vortexer to mix.

5. Make sure that the reagents are at the bottom of the tubes. If available, spin down the tube contents at 1000–2000 × g for approximately 30 seconds using a centrifuge with a plate adapter.
Start instrument run

This section describes the procedure for running the 8-tube reactions on the Applied Biosystems® 7500 Fast System using SDS software.

Run the 8-tube reactions

Use of the 7500 Fast Precision Plate Holder for MicroAmp® Tube Strips (Cat. no. 4403809) is recommended.

When you use the MicroAmp® 8-tube strips, if column 1 (far left) and column 12 (far right) are not used, insert two fully capped, empty, 8-tube strips into these columns.

Note: The empty capped 8-tube strips evenly distribute the clamping load applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes.

1. Carefully insert two or more 8-tube strips containing samples, starting from the center of the plate holder and moving out. This layout minimizes bending or misaligning the tube strips.

Note: A minimum of two and a maximum of twelve 8-tube strips can be run at one time.

IMPORTANT! Always use a total of 8 tubes per column. You may need to add new, empty tubes to a column.

2. Open the run file document that corresponds to the reaction plate that you created in “Create a run file document using SDS software” on page 19.
3. In the Instrument tab, select Start to begin the run.

**Note:** The run file must be saved before the run will start. If the run file was not saved before selecting Start then a message will appear informing you to save the run file.

**IMPORTANT!** To avoid false positives due to amplified material in your work area, do not open tubes after amplification.

## View results

### General process using SDS software

The general process for viewing results from MicroSEQ® *E. coli* O157:H7 Detection Kit involves:

- Viewing the amplification plots for all reactions.
- Setting the baseline and threshold values.
- Checking each sample for FAM™ dye (target-specific) signal and a NED™ dye (IPC) signal. The following table is a basic guide for interpreting the results:

<table>
<thead>
<tr>
<th>FAM™ dye signal (target 1)</th>
<th>VIC® dye signal (target 2)</th>
<th>NED™ dye signal (IPC)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+, –</td>
<td>Positive</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Inconclusive; no IPC detected</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Inconclusive; no IPC detected</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Inconclusive; no IPC detected and no target-specific signal detected</td>
</tr>
</tbody>
</table>

### Resources for viewing results

For more information about analyzing your data, refer to the appropriate instrument user guide.

We do not recommend using the same method to screen samples and to confirm the results. When you use the MicroSEQ® Pathogen Detection System to screen samples, culture and biochemical methods are recommended to confirm the result. Confirmation protocols include FSIS (USDA) MLG 5.04 and ISO 16654:2001 listed under “References” on page 51. Additional confirmation protocols are listed in Appendix A on page 40.
Kit workflow

This chapter presents a protocol for using the MicroSEQ® E. coli O157:H7 Detection Kit with the StepOne® software on the StepOnePlus® instrument.

**Isolate DNA**
- Pre-enrichment
- Sample preparation

**Prepare for PCR**
- Create a run file document using the StepOne® software
- Prepare the assay beads
- Prepare samples and controls
- Combine samples and controls with assay beads
- Prepare tubes for the StepOnePlus® System

**Start instrument run**
- Run the 8-tube reactions on the StepOnePlus® System

**View results on the StepOnePlus® System**

**IMPORTANT!** The MicroSEQ® E. coli O157:H7 Detection Kit can be used with the StepOnePlus® instrument, but not the StepOne® instrument, because the StepOne® instrument is unable to detect all three dyes in the E. coli O157:H7 assay.

**Note:** The StepOnePlus® instrument was not included in either the AOAC or AFNOR validation studies.
Isolate DNA

One of the following methods of isolating DNA is recommended with this detection kit. The following two documents describing these methods contain information on pre-enrichment and sample preparation using PrepSEQ® kits:

- **PrepSEQ® Nucleic Acid Extraction Kit User Guide: E. coli O157:H7** (automated magnetic bead-based protocol: Pub. no. 4426513)

<table>
<thead>
<tr>
<th>Kit</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrepSEQ® Nucleic Acid Extraction Kit – Food and Environmental Testing (includes optional Proteinase K)</td>
<td>4428176</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Kit ‡</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit</td>
<td>4407760</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit with Proteinase K</td>
<td>4426714</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean</td>
<td>4413269</td>
</tr>
<tr>
<td>PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K</td>
<td>4426715</td>
</tr>
</tbody>
</table>

‡ We recommend extra clean for matrices containing high fat content. Most food samples derived from animal sources show improved recovery of *E. coli* DNA with the addition of Proteinase K digestion, but no improvement was observed with non-animal products.
To prepare for PCR, first open the StepOne® software and create a run file document, then prepare the assay beads and samples. This chapter describes the basic procedure that uses StepOne® software for the StepOnePlus® Real-Time PCR System.

Create a run file document using StepOne® software

1. In the StepOne® software, create a run file document. Go to File ➤ New Experiment and select Advanced Setup.

   Note: For information on creating a run file document, refer to the documentation that is provided with your instrument.

2. In the Experiment Properties page, select the following:
   b. Type of experiment: Quantitation – Standard Curve
   c. Type of reagents: TaqMan® Reagents
   d. Ramp speed: Fast

3. In the Plate Setup page, under the Define Targets and Samples tab, create three targets with NFQ-MGB quenchers. Select FAM, VIC, and NED as the reporter dyes.
4. Under the Assign Targets and Samples tab, associate FAM™, VIC®, and NED™ detectors with each reaction.

**IMPORTANT!** Review “Start instrument run” on page 35 or “Run the 8-tube reactions on the StepOnePlus® System” on page 35, as appropriate, to determine the optimal layout to minimize bending or misaligning the tube strips. The MicroSEQ® *E. coli* O157:H7 Detection Kit kit can be used with the StepOnePlus® (but not the StepOne®) instrument.

*Note:* The FAM™ and VIC® dyes are used to detect the targets; the NED™ dye is used to detect the internal positive control (IPC). The *E. coli* O157:H7 assay is compatible with the StepOnePlus® instrument, but not the StepOne® instrument, because the StepOne® instrument is unable to detect all three dyes in the *E. coli* O157:H7 assay.

5. In the Run Method page, set thermal-cycling conditions as indicated in the following table. For more details, refer to “Chapter 5 Presence/Absence Experiments” in the *StepOne® and StepOnePlus® Real-Time PCR Systems User Guide* (Pub. no. 4379704).

<table>
<thead>
<tr>
<th>Step</th>
<th>Enzyme activation</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HOLD</td>
<td>Cycle (40 cycles)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Denature</td>
</tr>
<tr>
<td>Temp.</td>
<td>95°C</td>
<td>95°C</td>
</tr>
<tr>
<td>Time</td>
<td>2 min</td>
<td>1 sec</td>
</tr>
</tbody>
</table>
6. Set the Sample Volume to 30 µL.

The following image shows thermal cycling conditions with fast ramping for the StepOnePlus® instrument using StepOne® software:

7. Under File, save the run file as an .eds document in the appropriate folder.
Prepare for PCR

Prepare the assay beads

1. Open the storage pouch containing the assay beads (cut at the notch in the upper corner of the storage pouch above the zip-lock strip).

   **IMPORTANT!** Do not remove the desiccant from the storage pouch.

2. Remove the appropriate number of individual tubes or 8-tube strips based on the number of samples and controls that you plan to run, and place in a 96-well base. At least one negative control is recommended for the target pathogen. Remove colored caps and discard. Avoid disturbing the beads from the bottom of the tubes.

   **Note:** The assay beads contain all the components necessary for each reaction.

   **IMPORTANT!** Do not use colored caps or tubes for kit reactions. Colored caps or tubes may affect dye-signal readings during real-time PCR.

3. Seal the storage pouch using the zip-lock strip, then store at 5 ±3°C.
Prepare samples and controls

1. Thaw all reagents (samples and controls) completely.

2. Remove condensation after thawing samples and prior to opening to avoid cross contamination.
   - For PrepSEQ® Nucleic Acid Extraction Kit samples, centrifuge the plate at 1000–2000 × g for approximately 1 minute.
   - For PrepSEQ® Rapid Spin Sample Preparation Kit samples, microcentrifuge the tubes for at 12,000–16,000 × g for approximately 1 minute to bring down any particulate material derived from the spin column, which can interfere with amplification. The microbial DNA is in the aqueous phase.
   - For Pathogen Detection Negative Control and positive control(s) in microcentrifuge tubes, vortex the tubes, and then spin down.

   **Note:** Unknown samples and positive control samples are provided by the investigator. The kit includes a negative control (Pathogen Detection Negative Control).

3. Add 30 µL of sample prepared above to each assay bead. Dispense all unknown samples first, followed by negative control(s) and then positive control(s). Beads dissolve in 1–5 seconds. Mix by gently aspirating and dispensing a few times. (Alternatively, vortex assay tubes after they are capped in the final step.)

   **IMPORTANT!** Use a new pipette tip for each sample. Resuspend by gently pipetting up and down with the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination or, if a vortexer is available, vortex the capped tubes until the pellet is dissolved. See Appendix B, “Good PCR Practices” on page 43 for more information.

   **Note:** Add 30 µl of the PrepSEQ® Nucleic Acid Extraction Kit sample or 30 µL of the PrepSEQ® Rapid Spin Kit sample to each assay bead. The PrepSEQ® Nucleic Acid Extraction Kit provides ~100 µL of sample, and the PrepSEQ® Rapid Spin Kit provides 300 µL of sample. Use 30 µL of the Pathogen Detection Negative Control per negative control reaction. If you use other sample preparation methods, and less than 30 µL of sample is available, adjust the sample volume with water to 30 µL for each reaction type.
Prepare tubes for the StepOnePlus® System

8-tube strips containing assay beads are compatible with StepOnePlus® Systems.

1. For 8-tube strips with seven or fewer reactions, add additional empty tubes as needed so that each strip contains a full set of 8 tubes.

   **Note:** The empty capped 8-tube strips evenly distribute the clamping load that is applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes. Add empty tubes as needed to create a complete 8-tube strip and cap with an intact 8-cap strip.

2. Cap the tubes, sealing each tube with the transparent optical strip caps provided in the kit. Cap the tubes with the strip caps using the MicroAmp® 96-Well Base (Cat. no. N8010531) and the MicroAmp® Cap Installing Tool (Cat. no. 4330015) to avoid collapsing, bending, or misaligning the tubes. Confirm that the strips are straight and that each tube is in line with the adjacent tube.

3. Mark or label one end of the strip cap (but not over a cap) so that you will remember the orientation when transferring the tubes to the instrument tray.

4. Make sure that the reactions are thoroughly mixed. If reactions were not previously mixed during the pipetting step, place the assay tubes in a 96-well base and use a vortexer to mix.

5. Make sure that the reagents are at the bottom of the tubes. If available, spin down the tube contents at 1000–2000 × g for approximately 30 seconds using a centrifuge with a plate adapter.
Start instrument run

This section describes the procedure for running the 8-tube reactions on the StepOnePlus® Real-Time PCR System using StepOne® software.

Run the 8-tube reactions on the StepOnePlus® System

1. Place the MicroAmp® 96-Well Tray for Veriflex™ Blocks (Cat. no. 4379983) on the sample block of the StepOnePlus® System.

2. Load the 8-tube strips vertically (see the preceding figure). The minimum recommended load is two 8-tube strips (16 tubes), which you should place in adjacent columns, for example in columns 1 and 2. It is not necessary to balance the tube strips on the tray.

3. Open the run file document that corresponds to the reaction plate that you created in “Create a run file document using StepOne® software” on page 29.

4. Start the run.

**IMPORTANT!** To avoid false positives due to amplified material in your work area, do not open tubes after amplification.
View results

The general process for viewing results from MicroSEQ<sup>®</sup> E. coli O157:H7 Detection Kit involves:

- Viewing the amplification plots for all reactions.
- Setting the baseline and threshold values.
- Checking each sample for FAM™ dye (target-specific) signal and a NED™ dye (IPC) signal. The following table is a basic guide for interpreting the results:

<table>
<thead>
<tr>
<th>FAM™ dye signal (target 1)</th>
<th>VIC® dye signal (target 2)</th>
<th>NED™ dye signal (IPC)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+, −</td>
<td>Positive</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>−</td>
<td>Inconclusive; no IPC detected</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Inconclusive; no IPC detected</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Inconclusive; no IPC detected and no target-specific signal detected</td>
</tr>
</tbody>
</table>

Resources for viewing results

For more information about analyzing your data, refer to the appropriate instrument user guide.

We do not recommend using the same method to screen samples and to confirm the results. When you use the MicroSEQ<sup>®</sup> Pathogen Detection System to screen samples, culture and biochemical methods are recommended to confirm the result. Confirmation protocols include FSIS (USDA) MLG 5.04 and ISO 16654:2001 listed under “References” on page 51. Additional confirmation protocols are listed in Appendix A on page 40.
## Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>No IPC signal and no target-specific signal is detected in unknown wells.</td>
<td>Inhibition of PCR occurred.</td>
<td>Dilute the sample in water (for example, 1:5 or 1:10). Alternatively, repeat sample preparation to obtain a new sample for PCR.</td>
</tr>
<tr>
<td>(Note: When there is target-specific signal in the absence of IPC signal for an unknown sample, the result is considered positive and no troubleshooting is required.)</td>
<td></td>
<td><strong>Note</strong>: For more information, refer to the “Troubleshooting” section in the following:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• PrepSEQ® Nucleic Acid Extraction Kit for Food Testing User Guide: E. coli O157:H7 (Pub. no. 4426513)</td>
</tr>
<tr>
<td>No target-specific signal is detected in positive control wells.</td>
<td>A pipetting error occurred (no positive control was added).</td>
<td>Repeat the assay. Make sure to pipet positive control into all positive-control wells.</td>
</tr>
<tr>
<td>No IPC is detected, but target-specific signal is detected in unknown wells.</td>
<td>A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.</td>
<td>No action is required.</td>
</tr>
<tr>
<td>Target-specific signal is detected in negative-control wells.</td>
<td>Carryover contamination occurred.</td>
<td>Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. If the negative control continues to show contamination, repeat the assay using a new kit. If the negative control continues to show contamination, contact Life Technologies Technical Support.</td>
</tr>
<tr>
<td>No IPC is detected, but target-specific signal is detected in negative-control wells.</td>
<td>Carryover contamination and one of the following occurred:</td>
<td>Examine unknowns to determine if an IPC signal is present. If an IPC signal is present in unknown wells, IPC amplification is not a problem. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.</td>
</tr>
<tr>
<td></td>
<td>• A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• A problem occurred with IPC amplification.</td>
<td></td>
</tr>
<tr>
<td>No IPC signal is detected, but target-specific signal is detected in positive-control wells.</td>
<td>A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.</td>
<td>No action is required.</td>
</tr>
</tbody>
</table>
### Observation

| Amplicon contamination. | Contamination was introduced into the PCR clean area from post-amplification reaction tubes that were either opened in the clean area or brought into the PCR clean area from contaminated gloves or solutions. | To investigate possible amplicon contamination, prepare negative control samples (at least one 8-strip tube) using the MicroSEQ® Detection Kit lyophilized reaction mix.  
1. Divide the number of assay beads into two sets.  
a. To the first set of assay beads, add 30 µL of PCR-clean water  
b. To the second set of assay beads, add 29 µL of PCR-clean water plus 1 µL of 1 U/µL Uracil DNA Glycosylase (Cat. no. 18054-015).  
2. Run samples on the 7500 Fast instrument using SDS software and select the “Fast 7500” Run Mode.  
3. Under the instrument tab, select Add step to stage 1 of the PCR cycle that consists of 10 minutes at 37°C, and then extend the 95°C step from 20 seconds to 10 minutes.  
Results that show target-specific signal in the -UNG samples and no target-specific signal in +UNG samples is evidence of amplicon contamination.  
If the instrument block was contaminated, consult the 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide (Pub. no. 4347825) and/or contact a service representative to clean the instrument. |
| or | Contamination was introduced into the Real-Time PCR instrument from crushed and broken PCR reaction tubes. |

### Recommended action

1. Divide the number of assay beads into two sets.
   a. To the first set of assay beads, add 30 µL of PCR-clean water
   b. To the second set of assay beads, add 29 µL of PCR-clean water plus 1 µL of 1 U/µL Uracil DNA Glycosylase (Cat. no. 18054-015).
2. Run samples on the 7500 Fast instrument using SDS software and select the “Fast 7500” Run Mode.
3. Under the instrument tab, select Add step to stage 1 of the PCR cycle that consists of 10 minutes at 37°C, and then extend the 95°C step from 20 seconds to 10 minutes.

### Replicate results for this sample are inconsistent.

| All replicate wells for a sample do not have the same result. | If more than two replicates yield the same result (for example, you ran 3 replicates and 2 replicates are negative, but 1 replicate is positive), it is probable that the result with the larger number of replicates is accurate. However, your laboratory protocol may require that you repeat the assay using fresh samples and reagents.  
If you ran only two replicates and results are not consistent, repeat the assay using fresh samples and reagents. |
Appendix A

Background Information

Product overview

Description of target microorganisms

_Escherichia coli_ O157:H7 is a major foodborne pathogen that causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans. _E. coli_ O157:H7 is one of the main enterohemorrhagic _E. coli_ serotypes which secrete Shiga-like toxins. Outbreaks of _E. coli_ O157:H7 have been associated with contaminated food supplies such as raw ground beef, spinach, unpasteurized juices, and water.

Kit sensitivity

The limit of detection using this kit is $10^3$ colony-forming units (cfu)/mL.

The sensitivity of the assay in real culture samples depends on the quality of the sample preparation method that is used. The following protocols contain sample preparation procedures that allow you to detect 1–3 cfu in 25 grams, 25 mL, or 375 grams of food:

- PrepSEQ® Nucleic Acid Extraction Kit User Guide: _E. coli_ O157:H7 (Pub. no. 4426513)

Kit specificity

The MicroSEQ® _E. coli_ O157:H7 Detection Kit specifically detects the O157:H7 serotype.

Audience

This document is intended for investigators who need to test for _E. coli_ O157:H7 in food samples.
The MicroSEQ® *E. coli* O157:H7 Detection Kit earned the Performance Tested Methods℠ Certification from the AOAC Research Institute. The validation was conducted using USDA MLG 5.04 as the reference method for meat products, and ISO 16654 as the reference method for leafy green products and juices. The validated workflow includes:

- Two sample preparation kit options:
  - PrepSEQ® Nucleic Acid Extraction Kit
  - PrepSEQ® Rapid Spin Sample Preparation Kit
- MicroSEQ® *E. coli* O157:H7 Detection Kit
- Applied Biosystems® 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software

The workflow was certified for use with the following matrices:

- 25 g of ground beef and beef trim
- 375 g of ground beef and beef trim
- 25 g of spinach
- 25 g of apple juice
- 25 g of orange juice

The MicroSEQ® *E. coli* O157:H7 Detection Kit was certified “NF Validation”. The ISO 16140 standard was used for the validation of alternative methods. This kit was compared and found equivalent to the ISO 16654 reference method. The validated workflow includes:

- Two sample preparation kit options:
  - PrepSEQ® Nucleic Acid Extraction Kit
  - PrepSEQ® Rapid Spin Sample Preparation Kit
- MicroSEQ® *E. coli* O157:H7 Detection Kit
- Applied Biosystems® 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software

The method was certified for use with the following matrices: raw beef meat and raw produce. In the context of NF Validation:

1. Using workflows A (6-hour enrichment in BHI) and B (16-hour enrichment in BPW), test portions weighing more than 25 g have not been validated.

**Note:** For information about workflows A and B, see PrepSEQ® Nucleic Acid Extraction Kit for Food Testing User Guide: *E. coli* O157:H7 (Pub. no. 4426513) and PrepSEQ® Rapid Spin Sample Preparation Kits for Food Testing User Guide: *E. coli* O157:H7 (Pub. no. 4426519).
2. All samples identified as positive by the MicroSEQ® E. coli O157:H7 method must be confirmed by any of the following means:

- By streaking the enrichment broth on CT-SMAC or another selective medium for E. coli O157, such as Chrom ID. Any E. coli O157-like colonies must be plated onto Nutrient Agar, and after a 24-hour incubation, latex agglutination tests specific to both O157 and H7 must be performed with isolated colonies. If no characteristic colony is observed on the agar plates, proceed with an immunomagnetic separation (IMS) step prior to streaking 50 µL on selective agar (for example, by using Dynabeads® anti-E. coli O157 from 1 mL of the enrichment broth). Then, streak 50 µL onto the selective agar plate.

- According to classical tests described in methods standardized by CEN or ISO from colonies (including purification step).

- Any other method certified “NF Validation” based on a different principle than the MicroSEQ® E. coli O157:H7 method. It is necessary that the complete protocol for the second validated method be performed entirely, which means that the enrichment steps that precede the confirmation step must be common to both methods.

General recommendations:

- Comply with Good Laboratory Practices – GLP (Refer to EN ISO 7218 standard).

- ISO 16654 and ISO 7218 are recommended for preparation of master suspensions.

For more information about the expiration date of the “NF Validation” certification, please refer to the certificate, available on the website at: [www.afnor-validation.com](http://www.afnor-validation.com).

Operational conditions

The Applied Biosystems® 7500 Fast and StepOnePlus® Real-Time PCR Systems are for indoor use only and for altitudes not exceeding 2000 m (6500 ft) above sea level.

<table>
<thead>
<tr>
<th>Temperature and humidity requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Condition</strong></td>
</tr>
<tr>
<td>Temperature</td>
</tr>
<tr>
<td>Humidity</td>
</tr>
</tbody>
</table>
Definitions of terms

This protocol uses the following terms:

- **Amplification** – The process of making copies of, and thereby increasing the amount of, a specific DNA sequence.

- **Internal Positive Control (IPC)** – A control in all reaction wells that should always yield amplification, unless a high copy number of target DNA results in preferential amplification of the target DNA compared to the IPC. If the IPC and target DNA do not amplify, a problem with amplification exists. If the IPC signal is greatly reduced and the target DNA does not amplify, the unknown sample may contain a PCR inhibitor.

- **Negative control** – A reaction mixture that lacks a target sequence. It indicates contamination if amplification occurs, or an amplification problem if the IPC signal is reduced or absent. The Pathogen Detection Negative Control is provided in the kit as a negative control. At least one negative control is required for each target assay.

- **Polymerase chain reaction (PCR)** – Technology used to amplify, or increase the amount of a DNA sequence.

- **Positive control** – A control that establishes the expected amplification of a target. The lack of a target signal in a positive control well indicates a pipetting error or a problem with amplification. A positive control is provided by the investigator and is recommended but not required for each run.

- **Primer** – A segment of DNA that is complementary to the target DNA sequence or IPC DNA sequence. It is needed to start amplification.

- **Probe** – A segment of DNA that is complementary to the target DNA sequence or IPC DNA sequence. The TaqMan® probes contain both a fluorescent dye and a quencher dye. Upon binding to a specific sequence, and subsequent cleavage of the probe during the amplification step, the quencher is released and the reporter dye, no longer quenched, emits detectable fluorescence. The Sequence Detection System (SDS) or Real-Time PCR System detects the fluorescence, indicating the presence of the target or IPC DNA sequence.

- **Target** – The bacteria being tested.

- **Unknown sample** – A DNA sample from a food substance that you test for the presence of one or more food pathogens.
Prevent contamination and nonspecific amplification

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.

<table>
<thead>
<tr>
<th>PCR good laboratory practices</th>
<th>When preparing samples for PCR amplification:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).</td>
</tr>
<tr>
<td></td>
<td>• Change gloves whenever you suspect that they are contaminated.</td>
</tr>
<tr>
<td></td>
<td>• Maintain separate areas and dedicated equipment and supplies for:</td>
</tr>
<tr>
<td></td>
<td>– Sample preparation</td>
</tr>
<tr>
<td></td>
<td>– PCR setup</td>
</tr>
<tr>
<td></td>
<td>– PCR amplification</td>
</tr>
<tr>
<td></td>
<td>– Analysis of PCR products</td>
</tr>
<tr>
<td></td>
<td>• Never bring amplified PCR products into the PCR setup area.</td>
</tr>
<tr>
<td></td>
<td>• Open and close all sample tubes carefully. Try not to splash or spray PCR samples.</td>
</tr>
<tr>
<td></td>
<td>• Keep reactions and components capped as much as possible.</td>
</tr>
<tr>
<td></td>
<td>• Use a positive-displacement pipette or aerosol-resistant pipette tips.</td>
</tr>
<tr>
<td></td>
<td>• Clean lab benches and equipment periodically with 10% bleach solution, followed by a 70% ethanol rinse to remove residual bleach.</td>
</tr>
</tbody>
</table>

**IMPORTANT!** To avoid false positives due to cross-contamination:

• Prepare and close all negative-control and unknown sample tubes before pipetting the positive control.

• Do not open tubes after amplification.

• Use different sets of pipettors when pipetting negative-control, unknown, and positive-control samples.
Appendix B  Good PCR Practices
Prevent contamination and nonspecific amplification

Plate layout suggestions

- Separate different targets by a row if enough space is available.
- Put at least one well between unknown samples and controls if possible.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- Put positive controls in one of the outer rows or columns if possible.
- Consider that caps come in strips of 8 or 12.
WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.

- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
**Chemical safety**

**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant Safety Data Sheet (SDS) for specific precautions and instructions:

- Read and understand the SDSs provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!**

---

**Specific chemical handling**

<table>
<thead>
<tr>
<th>CAS</th>
<th>Chemical</th>
<th>Phrase</th>
</tr>
</thead>
<tbody>
<tr>
<td>26628-22-8</td>
<td>Sodium Azide</td>
<td>Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.</td>
</tr>
</tbody>
</table>
Biological hazard safety

**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: [www.cdc.gov/biosafety](http://www.cdc.gov/biosafety)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: [www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html](http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)
- Your company’s/institution’s Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: [www.cdc.gov](http://www.cdc.gov)

In the EU:

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining Certificates 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.

Obtaining 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
- 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): 1-800-500-6885
Phone number (Outside North America): Go to www.lifetechnologies.com/contactus.html and select the appropriate country from the drop-down menu.

Limited product warranty

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