

Real-Time PCR Detection of Shiga Toxin-Producing *E. coli* (STEC) in Beef Samples

USER GUIDE

Automated DNA isolation and real-time PCR detection of *E. coli* O157:H7 and "Big 6" non-O157 STEC

for use with:

PrepSEQ™ Nucleic Acid Extraction Kit

KingFisher™ Flex Purification System with 96 Deep-Well Head

MagMAX™ Express-96 Deep Well Magnetic Particle Processor

RapidFinder™ STEC Screening Assay

RapidFinder™ STEC Confirmation Assay

7500 Fast Real-Time PCR System

RapidFinder™ Express Software

Catalog Numbers 4480466, 4428176, 4476886, 4476901

Publication Number 4477013

Revision E



For testing of Food and Environmental samples only.

ThermoFisher
S C I E N T I F I C

Revision history: 4477013 E (English)

Revision	Date	Description
E	6 August 2024	<ul style="list-style-type: none">• Troubleshooting was added for possible instance of varying morphology of PCR pellets.• The software version was updated for RapidFinder™ Express Software.• Characteristics were added for the 7500 Fast Real-Time PCR Instrument.
D	1 March 2019	Information was added for the KingFisher™ Flex Purification System with 96 Deep-Well Head.
C	7 July 2016	<ul style="list-style-type: none">• Enrichment times were clarified for beef trim, 10–12 hours, and ground beef, 10–18 hours.• The storage conditions were changed for Magnetic Particles to 15–30°C.• The user guide template was updated with associated updates to the warranty information, trademark statements, and logos.• AOAC Research Institute <i>Performance Tested Methods</i>™ certification information was added.
B	25 July 2014	<ul style="list-style-type: none">• Enrichment incubation time increased from 8 hours to 10 hours (to allow detection of organisms that are potentially slow-growing or damaged by refrigerated storage).• The workflow schematic was adjusted to emphasize the importance of using prewarmed enrichment media.• The user guide template was updated with associated updates to the limited use label license information, warranty information, trademark statements, and safety statements.
A	1 December 2012	New document for RapidFinder™ STEC Detection Workflow (Automated DNA Isolation).

The information in this guide is subject to change without notice.

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IMPORTANT! Before using these products, read and understand the information in the "Safety" appendix in this document.

The RapidFinder™ Shiga toxin-producing *E. coli* (STEC) Detection Workflow is a complete protocol for the rapid presence/absence detection of *E. coli* O157:H7 and the “Big 6” non-O157 STECs (defined by the serogroups O26, O45, O103, O111, O121, and O145) in up to 375 g of ground beef or beef trim samples. The workflow consists of:

1. Enrichment of 375 g of ground beef or beef trim in prewarmed ($48 \pm 1^\circ\text{C}$) Tryptone Soya Broth (TSB).
2. Automated preparation of PCR-ready DNA using the Applied Biosystems™ PrepSEQ™ Nucleic Acid Extraction Kit and the KingFisher™ Flex Purification System with 96 Deep-Well Head, or the MagMAX™ Express-96 Deep Well Magnetic Particle Processor. The KingFisher™ Flex Purification System with 96 Deep-Well Head and the MagMAX™ Express-96 instrument enable high-throughput sample processing in a 96-well format with minimal handling.
3. Real-time PCR detection using the Applied Biosystems™ RapidFinder™ STEC Screening Assay and Applied Biosystems™ RapidFinder™ Express Software Version 2.0 or later. The screening assay includes probes and primers for *stx1*, *stx2*, *eae*, and *E. coli* O157:H7 gene targets.
 - Samples assessed as negative require no further testing.
 - Samples assessed as positive must be further tested with the Applied Biosystems™ RapidFinder™ STEC Confirmation Assay.
4. Real-time PCR detection using the RapidFinder™ STEC Confirmation Assay and RapidFinder™ Express Software Version 2.0 or later. This assay includes probes and primers for “Big 6” non-O157 STEC and *E. coli* O157:H7 targets, enabling:
 - Confirmation of the presence of a “Big 6” non-O157 STEC in samples that are positive for both *stx* and *eae* in the screening assay.
 - Confirmation of the presence of *E. coli* O157:H7 in samples that are positive for *E. coli* O157:H7 in the screening assay.

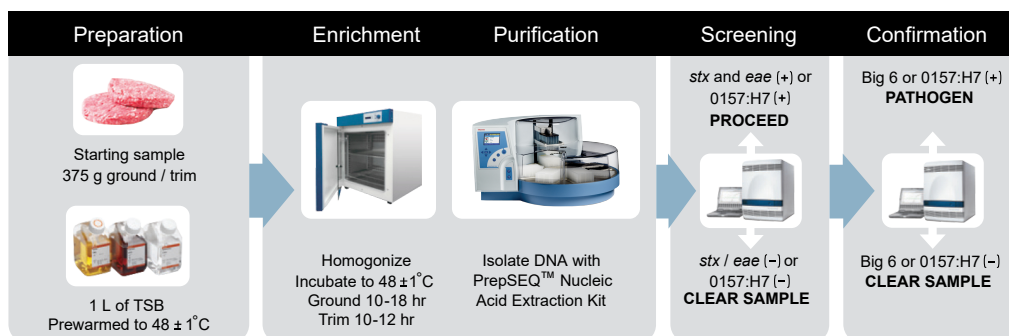


Figure 1 RapidFinder™ *E. coli* STEC detection workflow (automated method)

Up to 96 samples can be processed, from enrichment to real-time PCR screening assay results, in approximately 12 hours (beef trim samples) or 18 hours (ground beef samples); one additional hour is required for the real-time PCR confirmation assay.

Both RapidFinder™ STEC Assays are provided in a lyophilized assay bead format that includes:

- Probes and primers for the pathogen targets.
- An internal positive control (IPC) probe, primers, and template, to monitor for PCR inhibition.
- Enzyme and other buffer components necessary for real-time PCR.

A Pathogen Detection Negative Control is included in the assay kits. Unknown samples are provided by the investigator.

RapidFinder™ Express Software is a graphical, easy-to-use tool that provides step-by-step instructions to set up the real-time PCR assays on the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument followed by automated data analysis. Version 2.0 or later includes the STEC screening and STEC confirmation modules and must be used in this workflow. Online help is provided within the software.

The RapidFinder™ STEC Detection Workflow is intended for use by microbiological analysts who need to test for STEC in beef samples.

Visit <https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html> for a list of workflows for detection of *E. coli*.

Required materials

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or [thermofisher.com](https://www.thermofisher.com). They may also be available through Fisher Scientific ([fisherscientific.com](https://www.fisherscientific.com)), MLS, or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Note: Parts may ship separately depending on configuration and storage conditions.

Materials for enrichment of food samples

Item	Source
Tryptone Soy Broth (TSB) (Dehydrated)	CM0129
Enrichment bags with mesh, 10" × 15", 92 oz. (Whirl-Pak™ Filter Bag for Homogenizer Blenders or equivalent)	Nasco B01488WA or equivalent
Forced air incubators, 42±1°C and 48±1°C	MLS

Materials for DNA isolation

Table 1 PrepSEQ™ Nucleic Acid Extraction Kit

Contents	Cat. No. 4480466 (100 reactions)	Cat. No. 4428176 (300 reactions)	Storage ^[1]
Lysis Buffer	2 × 50 mL	6 × 50 mL	15°C to 30°C
Magnetic Particles	2 × 1.5 mL	6 × 1.5 mL	
Binding Solution (Isopropanol) ^[2]	1 empty bottle	3 empty bottles	
Wash Buffer Concentrate ^[3]	2 × 26 mL	6 × 26 mL	
Elution Buffer	25 mL	3 × 25 mL	
Proteinase K (PK) Buffer	50 mL	3 × 50 mL	
Proteinase K, 20 mg/mL	1.25 mL	3 × 1.25 mL	-25°C to -15°C

^[1] See the expiration date on the box.

^[2] Add ~35 mL of 100% isopropanol to the empty bottle before use.

^[3] Add 74 mL of 95% ethanol before use.

Table 2 Magnetic particle processor

Item	Source
KingFisher™ Flex-96 instrument and accessories	
KingFisher™ Flex Purification System with 96 Deep-Well Head	A32681 , 96 deep-well plate, or equivalent ^[1]
KingFisher™ 96 Deep-Well Plate, V-bottom	95040450
KingFisher™ 96 KF microplates (200 µL)	97002540
KingFisher™ Flex 96 heating block	24075420
KingFisher™ 96 tip comb for deep-well magnets	97002534
Fintip™ Filtered Pipette Tips	94052320 or equivalent
MagMAX™ Express-96 instrument and accessories	
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	Contact your local sales representative.
MagMAX™ Express-96 Deep Well Plates	4388476
MagMAX™ Express-96 Standard Plates	4388475
MagMAX™ Express-96 Deep Well Tip Combs	4388487

^[1] For the KingFisher™ Flex instrument, 96 plate with standard magnetic head (Cat. No. [5400620](#)), the 96 deep-well magnetic head is required (Cat. No. [24074430](#)).

Table 3 Other materials not included in the PrepSEQ™ Nucleic Acid Extraction Kit

Item	Source
Equipment	
96-Well Magnetic-Ring Stand	AM10050
Block heater, 37°C	MLS
Laboratory mixer (vortex or equivalent)	MLS
Pipettors: <ul style="list-style-type: none"> • Positive-displacement • Air-displacement • Multichannel 	MLS
<i>(Optional, but recommended)</i> Plate centrifuge	MLS
Consumables	
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS
<i>(Optional)</i> MicroAmp™ Clear Adhesive Film	4306311
Reagents	
Ethanol, 95%	MLS
Isopropanol, 100%	MLS
Nuclease-Free Water (not DEPC-Treated)	AM9938

Materials for PCR detection

Table 4 RapidFinder™ STEC Screening Assay (96 reactions; Cat. No. [4476886](#))

Contents	Amount	Color	Storage ^[1]
Lyophilized assay beads ^[2] , 8-tube strips	12 strips (96 tubes) 1 rack	Blue rack; blue, round-top, strip caps	5±3°C Protect from light and moisture. ^[3]
MicroAmp™ Optical 8-Cap Strips	12 strips (96 caps)	Clear	
Pathogen Detection Negative Control	1.5 mL	Red cap	5±3°C

^[1] See the product label for the expiration date.

^[2] Beads may have a purple-pink hue.

^[3] Store pouches in an airtight container.

Table 5 RapidFinder™ STEC Confirmation Assay (96 reactions; Cat. No. 4476901)

Contents	Amount	Color	Storage ^[1]
Lyophilized assay beads ^[2] , 8-tube strips	12 strips (96 tubes) 1 rack	Fuchsia rack; pink, round-top, strip caps	5±3°C Protect from light and moisture. ^[3]
MicroAmp™ Optical 8-Cap Strips	12 strips (96 caps)	Clear	
Pathogen Detection Negative Control	1.5 mL	Red cap	5±3°C

^[1] See the product label for the expiration date.

^[2] Beads may have a purple-pink hue.

^[3] Store pouches in an airtight container.

Table 6 Other required materials

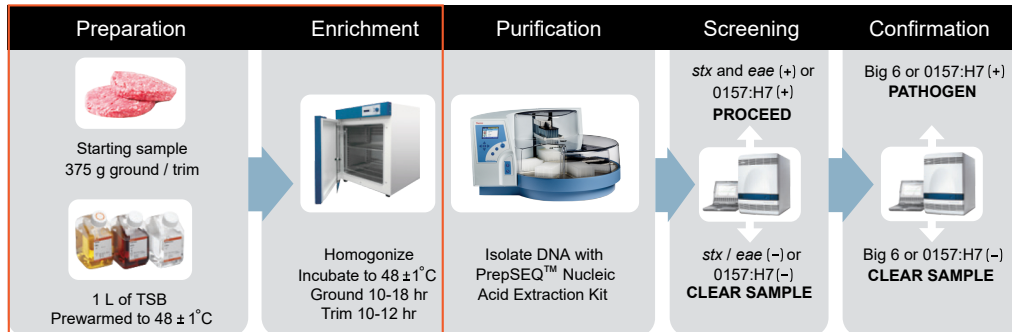
Item	Source
Real-time PCR instrument and accessories	
Applied Biosystems™ 7500 Fast Real-Time PCR System with RapidFinder™ Express Software Version 2.0 or later	A30304 (desktop) A30299 (laptop) Contact your local microbiology sales representative
MicroAmp™ 96-Well Base	N8010531
MicroAmp™ Cap Installing Tool	4330015
7500 Fast Precision Plate Holder, for 0.1 mL Tube Strips	A29252
Equipment	
Benchtop microcentrifuge with 8-tube strip adapter <i>or</i> Plate centrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS
Consumables and reagents	
Pipettors: <ul style="list-style-type: none"> • Positive-displacement • Air-displacement • Multichannel 	MLS
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS

Table 6 Other required materials *(continued)*

Item	Source
MicroAmp™ Fast 8-Tube Strip, 0.1 mL Fast 8-Tube Strip, 0.1 mL	4358293
MicroAmp™ Optical 8-Cap Strips (As needed for use as empty balance tubes)	4323032
Nuclease-Free Water (not DEPC-Treated)	AM9938

2

Homogenize and enrich food samples



IMPORTANT! Use proper aseptic technique while handling samples to avoid cross-contamination.

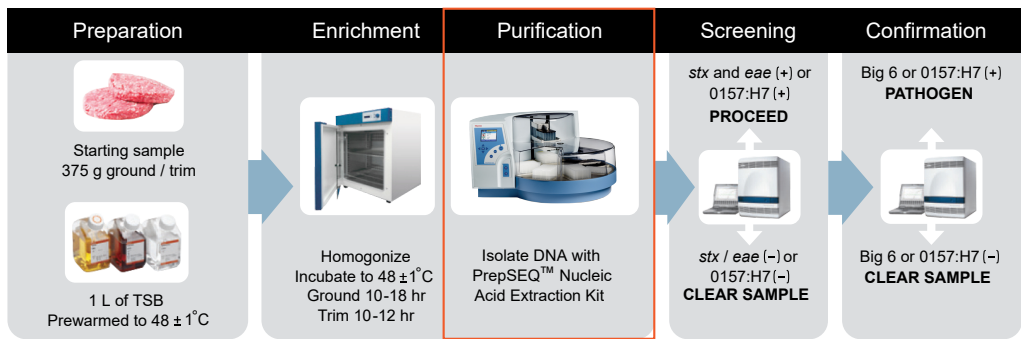
For each 375 g of beef sample:

1. Prepare 1 L of Tryptone Soya Broth (TSB) media.
2. Prewarm the TSB to 48±1°C.
3. Add 300–500 mL of prewarmed TSB to 375 g of beef sample in a filtered enrichment bag.
4. Close the bag and squeeze the bag at least 10 times.
This homogenizes the sample and distributes it evenly in the media.
5. Add the remaining prewarmed TSB to the bag, close the bag, and mix briefly by shaking or swirling.
6. Place the enrichment bag in a rack, ensuring sufficient space between bags to allow for air flow, and transfer the rack to a forced air incubator.
7. Incubate the samples at 42±1°C for:
 - Raw beef trim: 10–12 hours.
 - Raw ground beef: 10–18 hours.

3

Isolate DNA with the PrepSEQ™ Nucleic Acid Extraction Kit

Workflow



Important procedural guidelines

- If desired, prepare a negative extraction control: use media or water in place of the enriched culture in the DNA isolation procedure.
- For continuity with the downstream PCR, you can first set up the PCR run file in RapidFinder™ Express Software, then follow the Run Layout determined by the software to set up the Lysis Plate.

Before first use of the kit

Prepare Binding Solution and Wash Buffer

Before using a new PrepSEQ™ Nucleic Acid Extraction Kit, prepare the reagents:

- **Binding Solution**—Add approximately 35 mL of 100% isopropanol to an empty Binding Solution bottle. Label the bottle to indicate that isopropanol is added.
- **Wash Buffer**—Add 74 mL of 95% ethanol to the Wash Buffer Concentrate bottle, then mix well. Label the bottle to indicate that ethanol is added.

Before each use of the kit

Resuspend Magnetic Particles

IMPORTANT! Mix the particles vigorously before each use to ensure that all salts are dissolved.

White precipitate occasionally forms in the Magnetic Particles tube. Extraction experiments show that formation of precipitate does not affect performance as long as the precipitate is fully dissolved prior to use.

1. Incubate the tube of Magnetic Particles at 37±1°C for approximately 10 minutes.
2. Vortex for approximately 10 seconds.

Note: If the white precipitate is not completely dissolved after 10 minutes at 37°C, apply longer incubation times and higher temperatures (up to 50°C).

3. Keep at room temperature (23±5°C) until ready for use.

Prepare Proteinase K Buffer Mix

Combine the following components for the number of extractions required plus 10% overage, and mix well by inversion or vortexing.

Component	Volume per extraction
Proteinase K	10 µL
PK Buffer	200 µL
Total volume per extraction	210 µL

Use Proteinase K Buffer Mix immediately or store on ice until ready to use.

Prepare Binding Mix

Prepare Binding Mix just before use.

Combine the following components for the number of extractions required plus 10% overage, and mix well by vortexing for approximately 10 seconds.

Component	Volume per extraction
Binding Solution (isopropanol)	325 µL
Magnetic Particles ^[1]	25 µL
Total volume per extraction	350 µL

^[1] Resuspended and thoroughly mixed.

Set up the Lysis Plate

1. Distribute 210 µL of Proteinase K Buffer Mix to the designated sample wells in a Deep Well Plate.
2. Transfer 200 µL of enriched sample from the filtered side of the enrichment bag to a sample well.

Set up the processing plates

Add reagents to the processing plates as described in the following table:

Plate	Plate type	Action
Tip Comb	Standard	Place a 96-well Deep Well Tip Comb into a standard plate.
Elution Plate	Standard	Add 140 µL of Elution Buffer to each sample and control well.
Wash Plate 1	Deep well	Add 300 µL of Wash Buffer to each sample and control well.
Wash Plate 2	Deep well	Add 300 µL of Wash Buffer to each sample and control well.

Process samples on the instrument

1. Select the program on the instrument, and press **Start**.

Instrument	Program
KingFisher™ Flex-96	4476886PrepSEQ_STEC
MagMAX™ Express-96	4476886_PrepSEQ_STEC

2. Load the prepared plates according to the readout on the instrument.
Verify that the orientation of each plate to the rotating base is {A1 to A1}

Plate	Action
Tip Comb	Load the Tip Comb, then press Start .
Elution Plate	Load the Elution Plate, then press Start .
Wash Plate 1	Load Wash Plate 1, then press Start .
Wash Plate 2	Load Wash Plate 2, then press Start .
Lysis Plate	Load the Lysis Plate, then press Start .

3. Dispense Lysis Buffer when prompted by the instrument (after 10 minutes).
 - a. Remove the LysisPlate, and add 200 µL of Lysis Buffer to each sample and control well.
 - b. Load the Lysis Plate into the instrument, and press **Start**.
4. Dispense Binding Mix when prompted by the instrument (after 2 minutes).
 - a. Vortex the Binding Mix for 5–10 seconds to ensure uniform distribution of the Magnetic Particles.

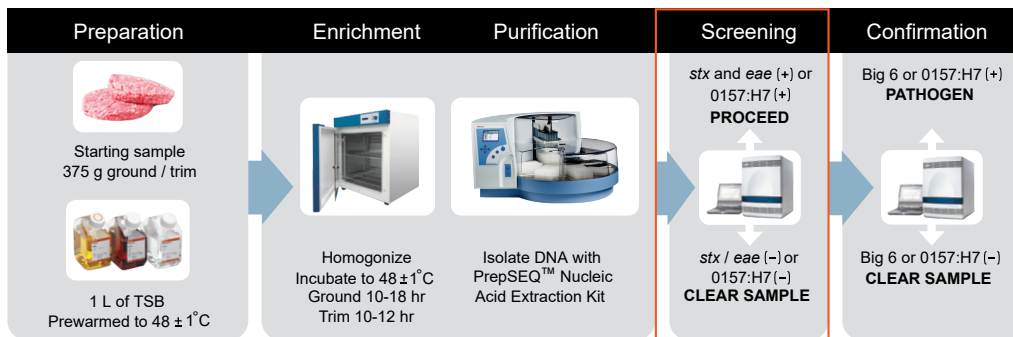
- b. Add 350 µL of Binding Mix to each sample and control well of the Lysis Plate.
 - c. Load the Lysis Plate into the instrument and press **Start**.
The remainder of the procedure is automated and does not require further user interaction.
5. When DNA sample preparation is complete (“Enjoy your DNA” is displayed on the screen), remove the Elution Plate from the instrument.
The Elution Plate contains approximately 130 µL of genomic DNA in Elution Buffer. It is normal for a small amount of Magnetic Particles to be present (brown pellet in the bottom of the sample well).
6. Proceed directly to real-time PCR, or seal the plate with MicroAmp™ Clear Adhesive Film and store the DNA in one of the following ways:
 - 5±3°C for up to 24 hours.
 - Below –18°C for long-term storage.

4

Perform PCR with the RapidFinder™ STEC Screening Assay

Screening workflow

In this step, real-time PCR is performed using the RapidFinder™ STEC Screening Assay and the PCR-ready DNA prepared with the PrepSEQ™ Nucleic Acid Extraction Kit, to initially assess samples for the presence of *stx1/2*, *eae*, and an indicator for the presence of *E. coli* O157:H7.



Important procedural guidelines for PCR assays

Software

- Ensure that you are using RapidFinder™ Express Software, Version 2.0 or later, which includes the STEC Screening and STEC Confirmation modules. The version number is displayed on the Main page of the software.
- Other pathogen modules cannot be selected when running STEC modules.
- The software determines the Run Layout (plate layout) during creation of the run file; therefore it must be set up before distributing DNA samples to the assay beads.

For additional information, refer to the *Applied Biosystems™ RapidFinder™ Express Software Quick Reference* (Pub. No. 4480999) or the online help within the software.

DNA sample collection for PCR

- If DNA samples were stored before PCR, thaw (if necessary), vortex, then centrifuge at 1,000–2,000 × *g* for approximately 1 minute to remove any condensation from the adhesive film before opening the plate (to avoid cross contamination).
- Avoid any particulate material or oils, when collecting the DNA sample for PCR.

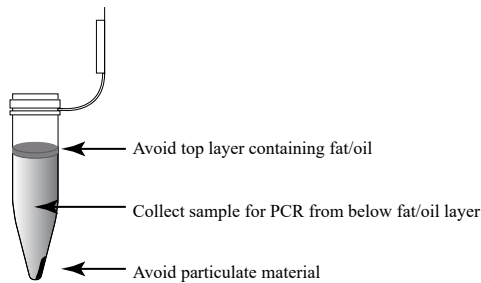


Figure 2 High-fat samples: collect sample from middle phase after lysis

If you see this in the Elution Plate...	Do this...
Oil droplets as a top layer	After lysis, food samples with high fat or oil content can form a top layer containing fat and debris over the aqueous phase containing the DNA. Collect the DNA sample for PCR from the clear middle phase, avoiding the top layer and bottom pellet (See Figure 2).
Magnetic Particles	<ol style="list-style-type: none"> 1. Place the Elution Plate on a 96-well magnetic ring stand for at least 1 minute. 2. Collect the eluate for PCR while the Elution Plate remains on the magnetic stand. Avoid touching the Magnetic Particles.
Particulate residue from food sample	<p>If the particulate residue is not removed using a 96-well magnetic ring stand:</p> <ol style="list-style-type: none"> 1. Centrifuge the Elution Plates at about 4000 × g for about 30 seconds in a plate centrifuge. 2. Avoid the particulate residue, and collect eluate for PCR.

Controls

- Use at least one negative control (Pathogen Detection Negative Control, provided with the RapidFinder™ kits) for each target assay.
- If desired, a negative extraction control, prepared during DNA isolation, can also be used.

PCR setup

IMPORTANT! Seal the tubes with the transparent, optical cap strips provided in the kit. Always use intact 8-cap strips, even if empty tubes have been added next to reaction tubes.

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

- Follow these instructions to ensure proper storage of the tube strips:
 - Cut the storage pouch at the notch above the resealable strip.
 - Always reseal the storage pouch with desiccant, and replace at 5±3°C.

- Follow these additional tips when setting up the PCR:
 - 8-tube strips can be cut apart with scissors.
If necessary, trim any remaining connector material from the cut to allow a better fit against adjacent tubes in the 7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips.
 - MicroAmp™ Tube Strips are labeled 1–8 on the side of the tubes to orient tube strips during handling.

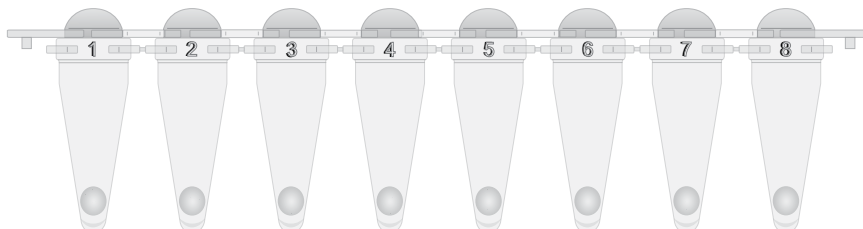



Figure 3 MicroAmp™ Tube Strip labeling The tube strip is shown with tinted dome caps, as shipped. For PCR, replace the dome caps with the optical cap strips provided in the kit.

If necessary for visual reference from above, mark the tab at one end of the cap strip. Do not mark any of the caps (this could interfere with real-time PCR detection).

- Use a new pipette tip for each sample.
- Use the MicroAmp™ 96-Well Base and the MicroAmp™ Cap Installing Tool to seal the assay tubes with the optical cap strips. This avoids collapsing, bending, or misaligning the tubes. Confirm that the strips are straight and that each tube is in line with the adjacent tube.
- Use a plate adapter for vortexing the tube strips, or hold the strips in the MicroAmp™ 96-Well Base while vortexing.
- Follow the recommendations in “Good laboratory practices for PCR” on page 31.

Create or edit the run file in RapidFinder™ Express Software

The software determines the Run Layout (plate layout) for your samples based on the information entered, and creates a run file.

On the main page of the RapidFinder™ Express Software, Version 2.0 or later, select **Create/Edit a Run File** , and enter or edit the Run File information at the prompts.

Select the **STEC Screen** module check box.

Refer to the online help in the RapidFinder™ Express Software for more details.

Prepare the assay beads

Follow the plate layout determined by the RapidFinder™ Express Software.

1. Transfer the appropriate number of individual tubes or 8-tube strips from the storage pouch to a 96-well base at room temperature (23±5°C).
2. If required by the plate layout, place empty MicroAmp™ Fast 8-Tube Strips (or partial strips) to balance the tray when the assay tubes are placed in the instrument later.

Set up the PCR reactions

Follow the guidelines in “DNA sample collection for PCR” on page 16 for samples that have been stored, or if the Elution Plate contains oil droplets, magnetic particles, or food particulate residue.

1. Following the plate layout determined by RapidFinder™ Express Software, add 30 µL of sample or control to each assay bead at room temperature (23±5°C).

Note: Maintain the layout and orientation of the tube strips in subsequent steps.

2. Seal the tubes with the transparent, optical cap strips provided in the kit.
3. Ensure that the reactions are thoroughly mixed: vortex at high speed for 5–10 seconds.
4. Ensure that the reagents are at the bottom of tubes: centrifuge the tubes at 1000–2000 × *g* for approximately 30 seconds.
5. Repeat step 3 and step 4.

IMPORTANT! Repeat the vortex/centrifugation steps to ensure complete mixing of the samples with the assay beads.

Load and run the reactions

In the RapidFinder™ Express Software, select **Start Instrument Run**  on the main page, select the appropriate run file, and follow the software prompts.

1. Transfer the tubes to the instrument in the same configuration as the run layout.
Use the 7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips in the instrument.
Be sure to load empty tube strips as directed by the software (Figure 4).
2. Close the tray to the instrument, and follow the RapidFinder™ Express Software prompts to start the run.

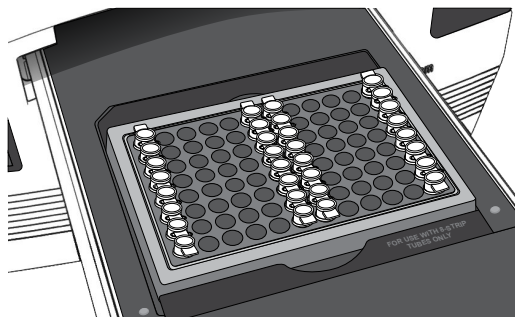


Figure 4 7500 Fast instrument tube layout RapidFinder™ Express Software directs the user to load empty strip tubes in column 1 (far left) and column 12 (far right), if needed. The empty capped 8-tube strips evenly distribute the clamping load applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes.

View screening assay results

RapidFinder™ Express Software automatically interprets the RapidFinder™ STEC Screening Assay results and generates a report that indicates which samples require subsequent testing with the RapidFinder™ STEC Confirmation Assay.

In RapidFinder™ Express Software, select **View Results** on the main page, select the appropriate run file, and follow the prompts to view results.

To display a list of results and assessment text in table format, click **Table View**. Select a sample, then click **View Details** to see replicate information about samples.

RapidFinder™ Express Software results icons

Result icon ^[1]	Result
+	Positive result
-	Negative result
!	Result warning

^[1] RapidFinder™ Express displays results pictorially.

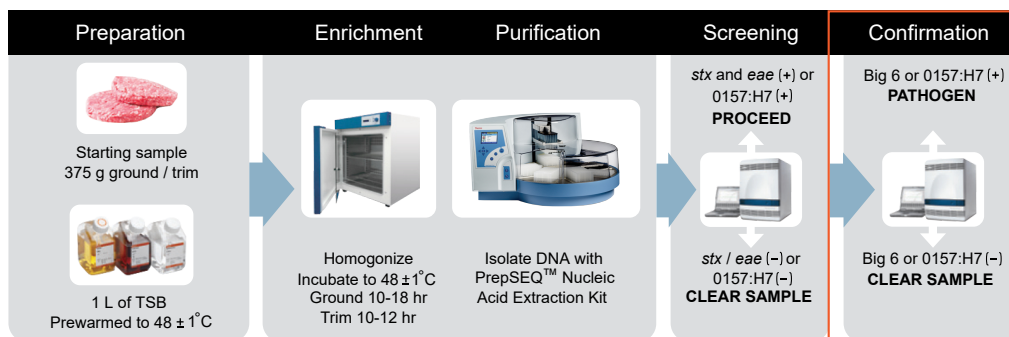
5

Perform PCR with the RapidFinder™ STEC Confirmation Assay

Confirmation workflow

In this step, real-time PCR is performed using the RapidFinder™ STEC Confirmation Assay and DNA samples that require confirmation testing:

- Samples that are positive for *stx* and *eae* in the screening assay, to confirm the presence of a “Big 6” non-O157 STEC.
- Samples that are positive for *E. coli* O157:H7 in the screening assay, to confirm the presence of *E. coli* O157:H7.



Set up and run the confirmation assay

1. Follow “Create or edit the run file in RapidFinder™ Express Software” on page 18, selecting the **STEC Confirm** module check box.
2. Proceed with “Prepare the assay beads” through 19.
The confirmation assays are in a fuchsia rack with pink-colored caps.

View, print, and export confirmation assay results

The RapidFinder™ Express Software automatically interprets the RapidFinder™ STEC Confirmation Assay results obtained during the real-time PCR run and generates a report based on the results of the confirmation assay and input data from the run setup of the screening assay.

Print or export results from the **Reports** tab of the **View Results** page.

Amplification curves may be viewed in SDS by clicking **View in SDS** on the **View Results** page.

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.

For more information about analyzing your data, see:

- Your instrument user guide.
- Online help within RapidFinder™ Express Software.



Confirmation methods

Recommended confirmation methods

In the context of AOAC Research Institute *Performance Tested Methods*SM certification, enriched cultures with positive results using the RapidFinder™ STEC Confirmation Assay were tested further by cultural confirmation using the appropriate reference method for the sample matrix (see “AOAC *Performance Tested Methods*SM Certification” on page 29).

Independent confirmation methods

The TaqMan™ assays listed in the following tables can be used to differentiate between serogroups in samples.

Note: These assays have not been validated as part of AOAC Research Institute *Performance Tested Methods*SM certification.

Table 7 TaqMan™ STEC assays (MLG design)

Assay ^[1]	Cat. No.
TaqMan™ STEC O103 & O145	4485063
TaqMan™ STEC O26 & O111	4485064
TaqMan™ STEC O45 & O121	4485065

^[1] Follow USDA FSIS MLG 5B (non-O157) guidelines.

Table 8 TaqMan™ STEC assays (ISO design)

Assay ^[1]	Cat. No.
TaqMan™ STEC 045 & 0121 Assay	4485082
TaqMan™ STEC 026, 0103 & 0145 Assay	4485083
TaqMan™ STEC 0111 & 0104 Assay	4485084

^[1] Follow EN ISO/TS 13136:2012 guidelines.



Troubleshooting

Observation	Possible cause	Recommended action
A visual difference in PCR beads is observed.	PCR pellets can exhibit differences in morphology.	Ensure thorough pipette mixing followed by vortexing on high speed to confirm pellet is in solution. After PCR, if IPC failure is observed, repeat the reaction.
Inhibition of downstream PCR, indicated by nondetection of IPC reaction	Magnetic Particles were in the Elution Plate.	Avoid disturbing the Magnetic Particles during transfer of eluted DNA to the lyophilized assay. Avoid transfer of Magnetic Particles using one of the following methods (optional): <ul style="list-style-type: none"> Place the Elution Plate on the 96-Well Magnetic Ring Stand during transfer of eluted DNA sample to the lyophilized assay. Spin the plate at maximum speed in a plate centrifuge for the equivalent of approximately $4,000 \times g$ for approximately 30 seconds, to pellet the Magnetic Particles to the bottom of the plate.
	Elution Plate contains incompletely removed particulate residue from the food sample.	Avoid residue during transfer of eluted DNA to the lyophilized assay. (Optional) Spin the plate at maximum speed in a plate centrifuge for the equivalent of approximately $4,000 \times g$ for approximately 30 seconds, to pellet the food residue to the bottom of the plate.
In positive control wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA existed in the samples, resulting in preferential amplification of the target-specific DNA.	No action is required. The result is considered positive.
In positive control wells, no target-specific signal is detected.	Positive control was omitted (pipetting error).	Repeat the assay. Make sure to pipette the positive control into all positive control wells.
In negative extraction control wells, target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred.	<ol style="list-style-type: none"> Repeat the assay using fresh aliquots of all reagents, fresh enrichment, and clean pipetting equipment. If the negative extraction control continues to show contamination, repeat the assay using a new kit. If the negative extraction control continues to show contamination, contact Technical Support.

Observation	Possible cause	Recommended action
<p>In negative control wells, no IPC signal is detected, but a target-specific signal is detected</p>	<p>Carryover contamination caused target signal in negative control wells.</p> <p>Additionally, no IPC signal in negative control wells could be caused by:</p> <ul style="list-style-type: none"> • A high copy number of target DNA existed in the samples, resulting in preferential amplification of the target-specific DNA. • A problem occurred with IPC amplification. 	<p>To correct carryover contamination, repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.</p>
		<p>To determine whether IPC amplification is a problem, examine unknown wells for an IPC signal. If an IPC signal is present, IPC amplification is not a problem.</p>
<p>In unknown wells, no IPC or target-specific signal is detected.</p>	<p>Inhibition of PCR occurred.</p>	<p>Dilute the sample 1:5 with nuclease-free water to dilute PCR inhibitors, then repeat the assay. If PCR remains inhibited, repeat the sample preparation.</p> <p>Refer to other troubleshooting suggestions for removal of Magnetic Particles or particulate residue from the DNA sample.</p>
<p>In unknown sample wells, no IPC is detected, but target specific signal ($C_T < 35$) is detected.</p>	<p>A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.</p>	<p>No action is required. The result may be considered positive.</p> <p>For some assays, both FAM™ and VIC™ targets must amplify. If only one amplifies, but the IPC does not, the result is considered inconclusive.</p>
<p>Replicate results for a sample are inconsistent.</p>	<p>All replicate wells for a sample did not have the same result.</p>	<p>If more than two replicates yield the same result (for example, 2 of 3 replicates are negative, but 1 replicate is positive), refer to your laboratory protocol to determine whether to repeat the assay using fresh samples and reagents.</p> <p>If only 2 replicates were run and the results are not consistent, repeat the assay using fresh samples and reagents.</p>

Observation	Possible cause	Recommended action
Amplicon contamination.	<ul style="list-style-type: none"> 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. Contamination was introduced into the real-time PCR instrument from crushed and broken PCR reaction tubes. 	<p>To confirm amplicon contamination, perform the following experiment:</p> <p>Prepare negative control samples using at least one 8-tube strip of MicroSEQ™ Assay Beads.</p> <ol style="list-style-type: none"> Divide the assay beads into two sets. <ol style="list-style-type: none"> To the first set of assay beads, add 30 µL of nuclease-free water. To the second set of assay beads, add 29 µL of nuclease-free water plus 1 µL of 1 U/µL Uracil DNA Glycosylase (Cat. No. 18054-015). Run samples on the 7500 Fast Real-Time PCR Instrument using SDS software, then select Fast 7500 run mode. Under the instrument tab: <ul style="list-style-type: none"> Select Add Step to stage 1 of the PCR cycle that consists of 10 minutes at 50°C. Extend the 95°C step from 20 seconds to 10 minutes. <p>Amplicon contamination is indicated by target-specific signal in the –UNG samples and no target-specific signal in +UNG samples.</p> <p>If the instrument block was contaminated, consult the <i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve</i> (Pub. No. 4347825) and/or contact a service representative to clean the instrument.</p>

Investigate warning results or failed runs in SDS Software

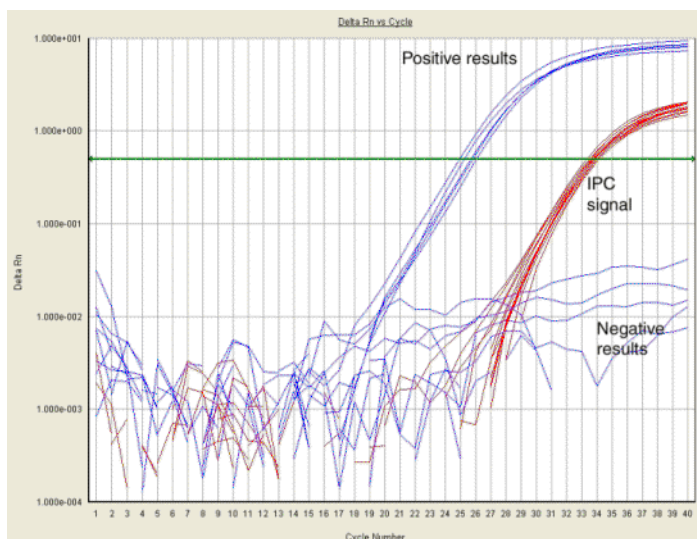
IMPORTANT! If you modify a RapidFinder™ Express Software run file in the SDS Software, you cannot open the run file again in the RapidFinder™ Express Software. To avoid altering a RapidFinder™ Express Software run file, save the run file under a new name in the SDS Software before performing any actions,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

- Open the run file in the SDS Software by one of the following methods:
 - From **View Results** in the RapidFinder™ Express Software, select and open the run file, then click **View in SDS**.
 - Open the run file in the SDS Software.
- Select **File ► Save As**, then save the run file under a new name.

3. Select the **Results** tab.
4. Select the **Amplification Plot** tab.
5. Select all locations by clicking the top left corner of the layout.

	1	2
A	N I	N I
B	U I	U I
C	N I	N I
D	U I	U I

6. Examine the Amplification Plot in Data mode of Delta Rn vs Cycle (displayed by default).



- To examine the signal for only tubes of interest, **Ctrl + Click** the locations below the plot.
- To examine the signal for only the IPC or a specific target, select the signal of interest from the Detector list at the top right of the plot.

Detector:

- Refer to “Interpretation of the amplification plot for samples with a Result Warning” on page 28.
7. When you finish viewing the run file, exit the SDS software:
 - If you accessed the run file from the RapidFinder™ Express Software, in the SDS Software, select **File ▶ Return to RapidFinder™ Express Software**.
 - If you opened the run file directly in the SDS software, in the SDS Software, select **File ▶ Exit**.



Interpretation of the amplification plot for samples with a Result Warning

For the IPC and the pathogen target detector, observe if the curve displayed in the Amplification Plot crosses the highlighted horizontal line, sometimes referred to as the "threshold" line.

IMPORTANT! The RapidFinder™ Express Software will automatically select the appropriate threshold values for each detector. Unless advised by a Thermo Fisher Scientific representative, do not change these values.

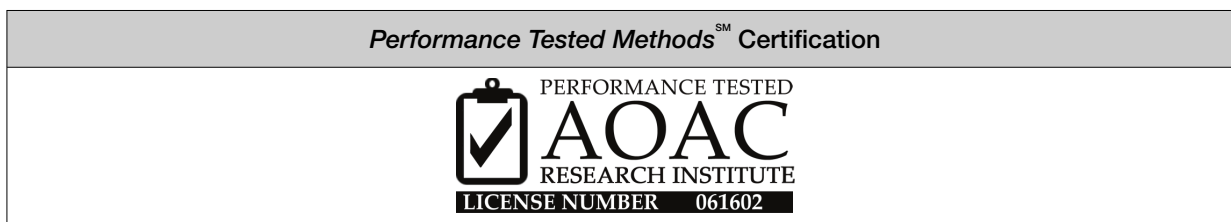
Additionally, the "highlighted horizontal line," as described above, will appear only when one of the detectors is selected. If "All" detectors are selected, the horizontal line will not be in the correct location for proper visual identification of the sample(s).



Supplemental information

AOAC Performance Tested MethodsSM Certification

Table 9 Performance Tested MethodsSM Certification of the workflow



The detection of *E. coli* O157:H7 and non-O157 STECs (defined by the serogroups O26, O45, O103, O111, O121, and O145) using the RapidFinderTM STEC Screening Assay and the RapidFinderTM STEC Confirmation Assay has earned the AOAC Performance Tested MethodsSM Certification from the AOAC Research Institute. The certified workflow described in this user guide includes:

- Enrichment in TSB
- PrepSEQTM Nucleic Acid Extraction Kit
- RapidFinderTM STEC Screening Assay and RapidFinderTM STEC Confirmation Assay
- Applied BiosystemsTM 7500 Fast Real-Time PCR Instrument
- RapidFinderTM Express Software Version 2.0 or later
- Applied BiosystemsTM 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 10 for characteristics) with RapidFinderTM Express Software Version 2.0 or later.

Table 10 7500 Fast Real-Time PCR Instrument characteristics

Characteristics	7500 Fast Real-Time PCR Instrument
Optics	12v 75w halogen bulb
Filters	5 excitation and 5 emission filters
Sample ramp rate	Standard mode: $\pm 1.6^{\circ}\text{C}/\text{sec}$ Fast mode: $\pm 3.5^{\circ}\text{C}/\text{sec}$
Thermal range	4-100°C
Thermal accuracy	$\pm 0.5^{\circ}\text{C}$
Thermal uniformity	$\pm 1^{\circ}\text{C}$
Format	96-well, 0.1-mL block

- Confirmation testing of positive samples using the reference method indicated in Table 11.

Table 11 Reference methods and validated matrices

Reference method	Matrix
USDA MLG 5.09 (<i>E. coli</i> O157:H7)	375 g of ground beef and beef trim
USDA MLG 5B.05 (<i>E. coli</i> non-O157 STECs)	

Go to <https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html> for a list of workflows for detection of *E. coli* (Pub. No. MAN0009419).

Sensitivity

The RapidFinder™ STEC Detection Workflow is certified to detect as few as 1–5 CFU of *E. coli* O157:H7 and/or “Big 6” non-O157 STECs (containing the genes encoding *stx* and *eae*) in a 375 g ground beef or beef trim sample enriched as indicated in this user guide.

Operational conditions

Enrichment for pathogenic *E. coli* should be performed in a laboratory certified to work with these organisms. Real-time PCR should be performed in a PCR-clean environment with good ventilation.

Table 12 Magnetic Particle Processor operation conditions (for indoor use only)

Condition	Acceptable range
Temperature	10–40°C
Humidity	Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C

Table 13 Applied Biosystems™ 7500 Fast Real-Time PCR Instrument operation conditions (for indoor use only)

Condition	Acceptable range
Temperature	10–40°C
Humidity	Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C
Altitude	Not exceeding 2,000 m (6,500 ft) above sea level

Good laboratory practices for PCR

Note: Spin tubes/plates before performing PCR. Spinning of PCR tubes is most easily accomplished by using a centrifuge designed for PCR tubes or plates. Follow manufacturer instructions for loading tubes/plates.

To avoid amplicon contamination of samples, follow these guidelines when preparing or handling samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
 - Sample preparation and reaction setup.
 - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Do not open reaction tubes after PCR.
- Do not autoclave reaction tubes after PCR.
- Clean lab benches and equipment periodically with 10% bleach solution or DNAZap™ Solutions (Cat. No. AM9890) according to the Thermo Fisher Scientific PCR Decontamination Protocol. After cleaning with bleach we recommend a rinse with distilled water or an ethanol solution because bleach will rust stainless steel. Note that minor discoloration of metal parts may occur.

For additional information, refer to EN ISO 22174:2005 or www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html.



Safety



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, and so on). To obtain SDSs, visit [thermofisher.com/support](https://www.thermofisher.com/support).



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. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.



- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311



Documentation and support

Food safety support

Website: <https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html> or [thermofisher.com/foodsafety](https://www.thermofisher.com/foodsafety)

Support email:

- Europe, Middle East, Africa: microbiology.techsupport.uk@thermofisher.com
- North America: microbiology@thermofisher.com

Phone: Visit [thermofisher.com/support](https://www.thermofisher.com/support), select the link for phone support, then select the appropriate country from the dropdown list.

Customer and technical support

Visit [thermofisher.com/support](https://www.thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.



Related documentation

Publication	Pub. No.
<i>Applied Biosystems™ RapidFinder™ Express Software Quick Reference</i>	4480999
<i>Applied Biosystems™ RapidFinder™ Express Software v1.2.1 and SDS software v1.4.2.1 User Bulletin</i>	4480998
<i>TaqMan™ Assays for Food and Environmental Testing User Guide</i>	MAN0009391

References

EN ISO 16654:2001. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157.

EN ISO 22174:2005. Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions.

EN ISO/TS 13136:2012. Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens – Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups.

U.S. Department of Agriculture, Food Safety and Inspection Service, Microbiology Laboratory Guidebook. Detection and isolation of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) from meat products and carcass and environmental sponges. Microbiology Laboratory Guidebook. MLG 5B.05.

U.S. Department of Agriculture, Food Safety and Inspection Service, Microbiology Laboratory Guidebook. Detection, isolation and identification of *Escherichia coli* O157:H7 from meat products and carcass and environmental sponges. Microbiology Laboratory Guidebook. MLG 5.08.

U.S. Department of Agriculture, Food Safety and Inspection Service, Microbiology Laboratory Guidebook. Detection, Isolation and Identification of *Escherichia coli* O157:H7 from meat products and carcass and environmental sponges. Microbiology Laboratory Guidebook. MLG 5.09.

