

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

USER GUIDE

Spin-column-based DNA isolation and real-time PCR detection of *E. coli* O157:H7 and “Big 6” non-O157 STEC

for use with:

PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K

RapidFinder™ STEC Screening Assay

RapidFinder™ STEC Confirmation Assay

7500 Fast Real-Time PCR System

RapidFinder™ Express Software

Catalog Numbers 4426715, 4476886, 4476901

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Revision A.0



For testing of Food and Environmental samples only.

ThermoFisher
SCIENTIFIC

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Revision	Date	Description
A.0	7 July 2016	New document. Includes the full workflow with AOAC™ Research Institute <i>Performance Tested Methods</i> ™ certification information.

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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. Spin-column-based preparation of PCR-ready DNA using the Applied Biosystems™ PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K.
3. Real-time PCR detection using the Applied Biosystems™ RapidFinder™ STEC Screening Assay and Applied Biosystems™ RapidFinder™ Express Software Version 1.2.1 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 1.2.1 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.

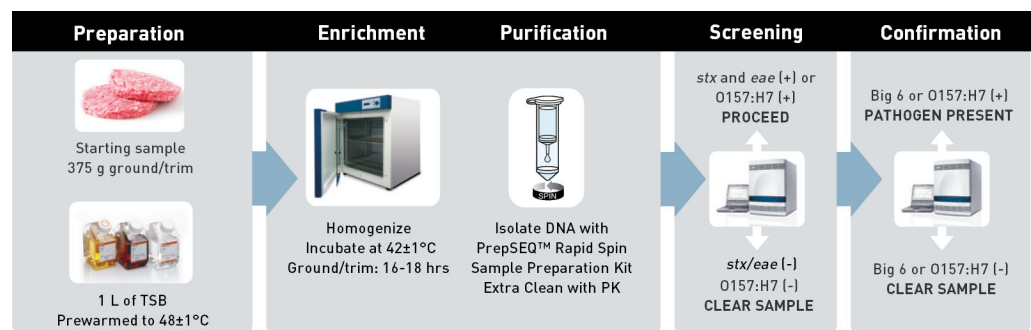


Figure 1 RapidFinder™ *E. coli* STEC detection workflow (spin column method)

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 1.2.1 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 thermofisher.com/foodsafety for a list of workflows for detection of *E. coli*.

Required materials

Unless otherwise indicated, all materials are available from Life Technologies (thermofisher.com). MLS: Fisher Scientific (www.fisherscientific.com) or other major laboratory supplier.

Note: Kit components may ship separately, depending on configuration and storage conditions.

Table 1 PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K (100 reactions, Cat. no. 4426715)

Components	Quantity or volume	Storage ^[1]
Spin columns	100	Room temperature (23±5°C)
Microcentrifuge tubes, 1.5 mL	2 × 100	
Lysis Buffer, 1 bottle	5 mL	5±3°C
Proteinase K (20 mg/mL), 1 tube	1.25 mL	Below -18°C

^[1] Refer to the product label for expiration date.

Table 2 RapidFinder™ STEC Screening Assay (96 reactions; Cat. no. 4476886)

Description	Amount	Storage ^[1]
Lyophilized assay beads ^[2] , 8-tube strips (blue caps)	12 strips (96 tubes) 1 rack	5±3°C Protect from light and moisture. ^[3]
MicroAmp™ Optical 8-Cap Strips (clear caps)	12 strips (96 caps)	
Pathogen Detection Negative Control (red cap)	1.5 mL	5±3°C

^[1] Refer to the product label for the expiration date.

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

^[3] Store pouches in an airtight container.

Table 3 RapidFinder™ STEC Confirmation Assay (96 reactions; Cat. no. 4476901)

Description	Amount	Storage ^[1]
Lyophilized assay beads ^[2] , 8-tube strips (pink caps)	12 strips (96 tubes) 1 rack	5±3°C Protect from light and moisture. ^[3]
MicroAmp™ Optical 8-Cap Strips (clear caps)	12 strips (96 caps)	
Pathogen Detection Negative Control (red cap)	1.5 mL	5±3°C

^[1] Refer to the product label for the expiration date.

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

^[3] Store pouches in an airtight container.

Table 4 Other required materials

Item	Source
Instrument and equipment	
Applied Biosystems™ 7500 Fast Real-Time PCR System with RapidFinder™ Express Software Version 1.2.1 or later	Cat. no. A30299, A30304
MicroAmp™ 96-well Base	Cat. no. N8010531
MicroAmp™ Cap Installing Tool	Cat. no. 4330015
7500 Fast Precision Plate Holder, for 0.1--mL tube Strips	Cat. no. A29252
For DNA isolation: Benchtop microcentrifuge	MLS

Item	Source
For PCR: Benchtop microcentrifuge with 8-tube strip adapter <i>or</i> Plate centrifuge	MLS
Forced air incubators, 42±1°C and 48±1°C	MLS
Block heaters, 97±2°C and 56±1°C	MLS
Laboratory vortex mixer	MLS
Rack for 1.5-mL tubes	MLS
Pipettors: <ul style="list-style-type: none"> • Positive-displacement • Air-displacement • Multichannel 	MLS
Consumables	
Enrichment bags with mesh, 10" × 15", 92 oz. (Whirl-Pak™ Filter Bag for Homogenizer Blenders, or equivalent)	Nasco B01488WA or equivalent
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS
As needed, for use as empty balance tubes: MicroAmp™ Fast 8-Tube Strip, 0.1 mL MicroAmp™ Optical 8-Cap Strips	Cat. no. 4358293 Cat. no. 4323032
Reagents	
Nuclease-Free Water	Cat. no. AM9938
Tryptone Soya Broth, dry media	Oxoid CM0129



Homogenize and enrich food samples

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

1. For each 375 g of beef sample, prewarm 1 L of prepared Tryptone Soya Broth (TSB) to $48\pm 1^\circ\text{C}$.
2. Combine 300–500 mL of prewarmed TSB with 375 g of beef sample in a filtered enrichment bag, and squeeze the bag at least 10 times to homogenize the sample.
3. Add the remaining prewarmed TSB to the bag, close the bag, and mix briefly by shaking or swirling.
4. 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.
5. Incubate the samples at $42\pm 1^\circ\text{C}$ for 16–18 hours under static conditions.

3

Isolate DNA using the PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K

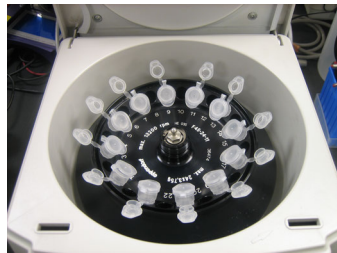
Procedural guidelines

PCR-clean water

Use Nuclease-Free Water (Cat. no. AM9938) for all procedures described in this protocol that require water. Nuclease-Free Water is considered “PCR-clean” water. However, autoclaved water should not be considered PCR-clean water.

Position of the spin column/tube assembly in the microcentrifuge

Place the tube cap hinge toward the inside of the rotor, and position the cap in the opposite direction of rotation.



Incorrect position of tube caps



Correct position of tube caps

For high-fat samples: remove fat layer before lysis

For samples that contain a distinct, top, fat layer following centrifugation, remove the fat layer and supernatant as follows:

Type of fat layer	Fat layer and supernatant removal
Liquid	<ol style="list-style-type: none"> 1. Use a P1000 pipettor to remove fat from the top surface by aspirating in a circular motion without disturbing the pellet. 2. Continue to collect supernatant from the top surface until all the supernatant is removed. 3. Discard the supernatant into a waste container.
Solid	<ol style="list-style-type: none"> 1. Use a pipettor to gently dislodge the fat layer without disturbing the pellet. 2. Aspirate the supernatant from the top surface using a pipettor until all the supernatant is removed. 3. Discard the supernatant into a waste container.

Before each use of the kit

- Prepare Proteinase K-Lysis Buffer: combine the following components for the number of samples required; store on ice until use.

Component	Volume per sample	Volume for n samples ^[1]
Proteinase K, 20 mg/mL	5 μ L	5.5 μ L $\times n$
Lysis Buffer	50 μ L	55 μ L $\times n$

^[1] Includes 10% overage.

- Preheat block heaters to 97 \pm 2°C and 56 \pm 1°C.

Filter 750 μ L of enriched culture through the spin column

Gently mix the enriched culture before transferring the sample to the spin column.

1. Insert a spin column into a labeled tube, transfer 750 μ L of the enriched sample from the filtered side of the enrichment bag to the spin column, and cap the column.

2. Microcentrifuge the spin column assembly at 12,000–16,000 $\times g$ for about 3 minutes.

Follow “Position of the spin column/tube assembly in the microcentrifuge” on page 10.

3. Remove the assembly from the microcentrifuge and discard the used spin column.

4. Gently aspirate the supernatant without disturbing the pellet, then discard the supernatant.

To remove liquid on the sides of the tube, push droplets into the supernatant by circling the inside of the tube with the pipettor before aspiration.

(Optional) If necessary, follow “For high-fat samples: remove fat layer before lysis” on page 10

Lyse the sample

1. Add 55 μ L of Proteinase K-Lysis Buffer to the pellet, and pipet up and down or vortex until the pellet is well dispersed.

2. Transfer the mixture to a clean 1.5-mL tube, avoiding residual fat.

The pellet must be well dispersed in the Lysis Buffer prior to transfer.

Avoid contact with residual fat on the sides of the original tube, and transfer only the Lysis Buffer containing the resuspended pellet.

3. Cap the tube, then incubate at 56 \pm 1°C for at least 30 minutes.

4. Incubate at $97\pm 2^{\circ}\text{C}$ for 12 ± 2 minutes, then allow the sample to cool for about 2 minutes at room temperature ($23\pm 5^{\circ}\text{C}$).
5. Microcentrifuge the tube at $12,000\text{--}16,000 \times g$ for about 1 minute to collect the contents at the bottom of the tube.
6. Add 250 μL of Nuclease-free Water, and mix thoroughly.
7. Microcentrifuge the tube at $12,000\text{--}16,000 \times g$ for 1–2 minutes to pellet any remaining particulate material.
The microbial DNA is in the supernatant.

Proceed directly to real-time PCR. Alternatively, store the DNA in one of the following ways:

- At $5\pm 3^{\circ}\text{C}$ for up to 24 hours.
- Below -18°C for up to 1 year.

If required, validate storage of the DNA according to ISO 20837 (2006).

4

Perform PCR with the RapidFinder™ STEC Screening Assay

Important procedural guidelines for PCR assays

Software

- Ensure that you are using RapidFinder™ Express Software, Version 1.2.1 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.

Sample handling

- If DNA samples have been stored or the pellet has dispersed, thaw the samples (if necessary), vortex, and centrifuge at 12,000–16,000 × g for 1–2 minutes. This step will avoid cross-contamination and exclude particulate matter from the PCR.
- Use a new pipette tip for each sample.
- If you mix the assay beads with the DNA samples by pipetting up and down, keep the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination.
- Follow the recommendations in “Good laboratory practices for PCR and RT-PCR” on page 14.

For high-fat samples after lysis: collection of DNA sample for PCR

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.

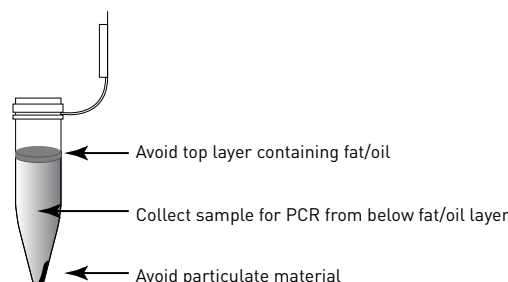


Figure 2 High-fat samples: collect sample from middle phase after lysis.

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.

MicroAmp™ tube strips

- 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 $5\pm 3^{\circ}\text{C}$.
- 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.

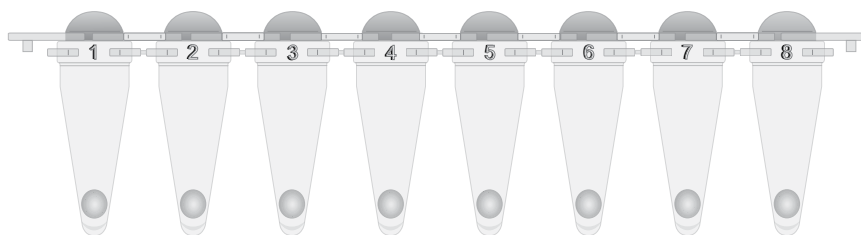


Figure 3 MicroAmp™ Tube Strip labeling

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).

- Seal the tubes with the transparent, optical cap strips provided in the kit. Do not use colored caps or tubes for real-time PCR reactions, because they may affect dye-signal readings during real-time PCR.
- Always use intact 8-cap strips, even if empty tubes have been added next to reaction tubes.
- 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.

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-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.
- Clean lab benches and equipment periodically with 10% bleach solution or DNAZap™ Solutions (Cat. no. AM9890).

For additional information, refer to ISO 22174 (2005).

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 1.2.1 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 “Sample handling” on page 13 for samples that have been stored.

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.
2. Close the tray to the instrument, and follow the RapidFinder™ Express Software prompts to start the run.

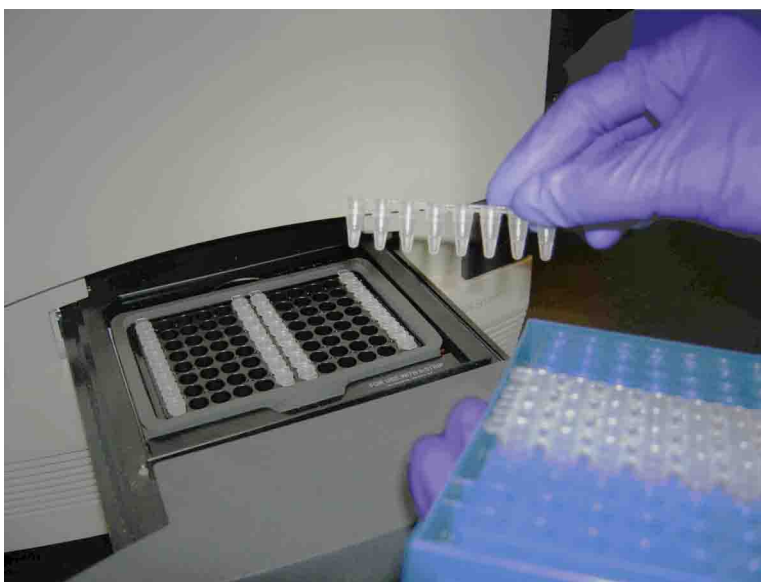


Figure 4 7500 Fast instrument tube layout

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.



Perform PCR with the RapidFinder™ STEC Confirmation Assay

Set up and run the confirmation assay

1. Follow “Create or edit the run file in RapidFinder™ Express Software” on page 15, selecting the **STEC Confirm** module check box.
2. Proceed with “Prepare the assay beads” through 16.
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.

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™ Research Institute *Performance Tested Methods*SM certification” on page 25).

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 5 TaqMan^{fi} STEC assays (MLG design)

Assay [1]	Cat. no.
TaqMan ^{fi} STEC 0103 & 0145	4485063
TaqMan ^{fi} STEC 026 & 0111	4485064
TaqMan ^{fi} STEC 045 & 0121	4485065

[1] Follow USDA FSIS MLG 5B (non-0157) guidelines.

Table 6 TaqMan^{fi} STEC assays (ISO design)

Assay [1]	Cat. no.
TaqMan ^{fi} STEC 045 & 0121 Assay	4485082
TaqMan ^{fi} STEC 026, 0103 & 0145 Assay	4485083
TaqMan ^{fi} STEC 0111 & 0104 Assay	4485084

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



Troubleshooting

General troubleshooting

Observation	Possible cause	Recommended action
The PCR was inhibited, as indicated by non-detection of the IPC reaction.	Removal of the supernatant was insufficient before addition of Lysis Buffer.	Dilute the sample 1:5 or 1:10 with Nuclease-free Water to dilute PCR inhibitors. If PCR remains inhibited, repeat the sample preparation.
	Filtrate from the spin column is in the sample.	Centrifuge the sample to separate the filter particulates before transferring sample to the PCR .
	Excess fat was not removed during aspiration of the supernatant.	Apply PrepSEQ™ Rapid Spin extra clean protocol.
	The sample matrix is associated with PCR-inhibitory components.	Pre-wash the bacterial pellet before loading the Rapid Spin column: <ol style="list-style-type: none"> 1. Transfer 750 µL of sample to a clean microcentrifuge tube. 2. Centrifuge at 12,000–16,000 × <i>g</i> for about 3 min. 3. Discard supernatant. 4. Resuspend pellet in 650 µL of sterile distilled water. 5. Load the resuspended sample onto the spin column.
In positive control wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA exists in 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 pipet the positive control into all positive control wells.

Observation	Possible cause	Recommended action
In negative control wells, target-specific signal is detected.	Carryover contamination occurred.	<ol style="list-style-type: none"> 1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 2. If the negative control continues to show contamination, repeat the assay using a new kit. 3. If the negative control continues to show contamination, contact Technical Support.
In negative control wells, no IPC signal is detected, but a target-specific signal is detected.	<p>Carryover contamination caused target signal in negative control wells.</p> <p>Additionally, no IPC signal in negative control wells can be caused by:</p> <ul style="list-style-type: none"> • A high copy number of target DNA exists in 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>
In unknown wells, no IPC or target-specific signal is detected.	Inhibition of PCR occurred.	<p>Dilute the sample 1:5 with Nuclease-free Water to dilute PCR inhibitors, and repeat the assay. If PCR remains inhibited, repeat the sample preparation.</p> <p>Refer to other troubleshooting suggestions for removal of particulates from the DNA sample.</p>
In unknown sample wells, no IPC is detected, but target specific signal ($C_T < 35$) is detected.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.	<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>
Replicate results for a sample are inconsistent.	All replicate wells for a sample do not have the same result.	<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 and 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>7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide</i> (Pub. no. 4347825) and/or contact a service representative to clean the instrument.</p>

Investigate warning results or failed runs in SDS Software

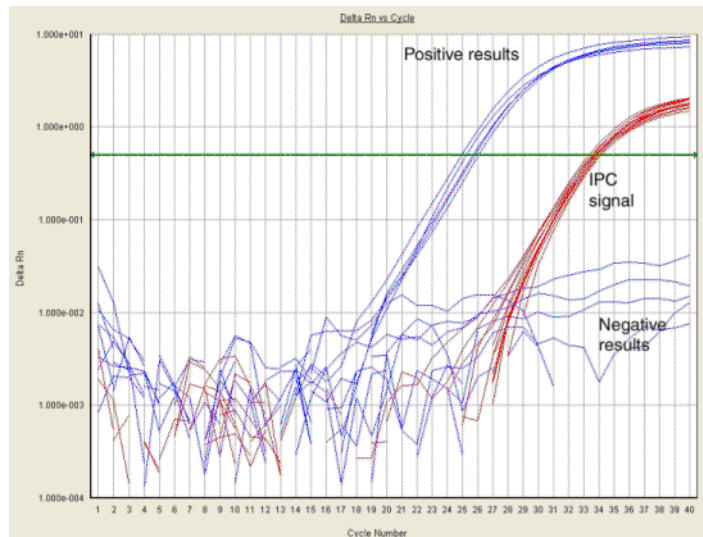
IMPORTANT! If you modify a RapidFinder™ Express Software run file in the SDS Software, you cannot open the run file again in the RapidFinder™ Express Software. To avoid altering a RapidFinder™ Express Software run file, save the run file under a new name in the SDS Software before performing any actions, 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.
- 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 24.
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™ Research Institute *Performance Tested Methods*™ certification



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

- Enrichment in TSB
- PrepSEQ™ Nucleic Acid Extraction Kit
- RapidFinder™ STEC Screening Assay and RapidFinder™ STEC Confirmation Assay
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software Version 1.2.1 or later
- Confirmation testing of positive samples using the reference method indicated in Table 7.

Table 7 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)	

Visit thermofisher.com/foodsafety for a list of workflows for detection of *E. coli* (Pub. no. MAN0009419).

Sensitivity

The RapidFinder™ STEC Detection Workflow is validated 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.

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-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.
- Clean lab benches and equipment periodically with 10% bleach solution or DNAZap™ Solutions (Cat. no. AM9890).

For additional information, refer to ISO 22174 (2005).



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, 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. 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 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!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.
-



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. 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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf
 - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
-

Documentation and support

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^{fi} Assays for Food and Environmental Testing User Guide</i>	MAN0009391

Customer and technical support

Visit thermofisher.com/support for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
 - Product FAQs
 - Software, patches, and updates
- Order and web support
- Product documentation, including:
 - 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.

Food Safety support

Website: thermoscientific.com/foodmicro or thermofisher.com/foodsafety

Support email:

- For products ordered through the Microbiology Division:
 - Europe, Middle East, Africa: microbiology.ts.eu@thermofisher.com
 - North America: microbiology.ts.us@thermofisher.com
- For products ordered through the Life Sciences Division:
foodsafety@lifetech.com

Phone: Visit **[thermofisher.com/support](http://www.thermofisher.com/support)**, select the link for phone support, and select the appropriate country from the dropdown menu.

Limited product warranty

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References

- ISO. 2001. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157. Reference number 16654:2001.
- ISO. 2005. Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – General requirements and definitions. Reference number 22174:2005.
- ISO. 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. Reference number ISO/TS 13136:2012(E).
- 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.

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