

SureTect™ *Escherichia coli* O157:H7 PCR Assay USER GUIDE

Lysis and real-time PCR detection of *Escherichia coli* O157:H7 in
food samples

for use with:

Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument with
Thermo Scientific™ RapidFinder™ Analysis Software v1.1 or later

Applied Biosystems™ 7500 Fast Real-Time PCR Instrument with Applied
Biosystems™ RapidFinder™ Express Software v2.0 or later

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Revision	Date	Description
C.0	13 July 2020	Updated software versions.
B.0	03 February 2020	Added enrichment conditions for 25 g fruits and vegetables. Added information about using the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument.
A.0	10 January 2019	Initial release with new publication number. Supersedes Pub. No. D12162 Version 5. Additional changes: <ul style="list-style-type: none">• Includes the complete NF VALIDATION™ by AFNOR Certification workflow that covers enrichment, DNA isolation, and real-time PCR detection.• Added Windows™ 10 software support.• Corrected assay kit file references.• Added a mixing step to ensure pellet is re-hydrated, in "Set up the PCR reactions."• Removed information about the PikoReal™ Real-Time PCR System.• Updated to the current document template, with associated changes in document organization, licensing, trademarks, and logos.

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For testing of Food and Environmental samples only.

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Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

Name and intended use

The Thermo Scientific™ SureTect™ *Escherichia coli* O157:H7 PCR Assay enables real-time PCR detection of *Escherichia coli* O157:H7 from food samples. These kits are for use in laboratories undertaking microbiological analysis, and they are compatible with the following instruments and software:

PCR instrument	Software	Pathogen Assay File
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument	Thermo Scientific™ RapidFinder™ Analysis Software v1.1 or later	E.coliO157H7_SureTect_QS5 version 2.0 or later ^[1]
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument	Applied Biosystems™ RapidFinder™ Express Software v2.0 or later	E.coliO157H7 SureTect 2.0 or later ^[1]

^[1] Assay files and instructions are available at thermofisher.com/rapidfinder-analysis-software.

Principle of the test

This assay is based upon use of Solaris™ reagents for performing PCR. Dye-labeled probes target unique DNA sequences specific to *Escherichia coli* O157:H7, and an internal positive control (IPC). Target DNA, if present, is detected by real-time PCR. Analysis software provides interpretation of results. For more information about real-time PCR, go to thermofisher.com/qpcducation.

The IPC template, primers, and probe provide an internal control with each reaction to show that the PCR process has occurred. It is unnecessary to incorporate positive control organisms with routine testing of samples.

Procedure overview

Enriched food samples are combined directly with ready-to-use Lysis Reagent 1 and Proteinase K, to lyse bacterial cells present in the sample and release their DNA into solution.

Lysates are transferred to the SureTect™ *Escherichia coli* O157:H7 PCR Tubes to rehydrate the lyophilized PCR pellets. The pellets contain lyophilized target-specific primers, dye-labelled probes, and PCR master mix components. The PCR tubes are sealed, loaded into the real-time PCR instrument, then the run is started using the RapidFinder™ software. After the run is complete, the software displays the interpreted results as simple positive or negative symbols. The results can be reported, stored, printed, and downloaded as required.

Results are achieved approximately 80 minutes after loading the prepared sample into the instrument.

Limitations

- The test is designed to detect DNA from target organisms that have been present at a minimum level of 1 CFU/sample, and have grown to detectable levels during the enrichment.
- The customer is responsible for validation of sample matrices or culture media not described in this document.
- When testing a sample type or culture medium that has not been validated, we recommend testing a selection of known negative and positive samples, to ensure that expected results are achieved. See “Test control organisms” on page 25 and EN ISO 22174:2005.
- See Appendix A, “Troubleshooting” for additional information.

Contents and storage

Store the kit protected from light, at 2–8°C. Bring to room temperature before opening.

Table 1 SureTect™ Escherichia coli O157:H7 PCR Assay, 96 tests (Cat. No. PT0400A)

Contents	Amount
Lysis Reagent 1 Tubes (clear, pale blue liquid containing fine white particles)	12 strips of 8 tubes
Lysis Tube Caps, domed	12 strips of 8 caps
Proteinase K (clear colorless liquid)	1 tube
SureTect™ Escherichia coli O157:H7 PCR Tubes	12 strips of 8 tubes 1 pellet each
PCR Caps	12 strips of 8 caps

Required materials

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

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

Materials for enrichment

Table 2 Equipment, accessories, and consumables

Item	Source
Homogenizer Laboratory Blender or Dilutor, one of the following, or equivalent	DB5000A DB4100A DB4150A
Homogenizer bags appropriate for the sample type and size	For DB4100A or DB4150A: DB4011A DB4012A DB4013A DB4014A
Incubator fitted with racks for homogenizer bags, set to 41.5±1°C	thermofisher.com
Disposable gloves	MLS
Variable volume single-channel pipette, 1- to 10-mL	Available through the Thermo Fisher Microbiology ordering process.
96-well rack	
Filtered pipette tips, 1- to 10-mL	
Sample tubes, 1.5-mL	

Table 3 Media

Item	Source
Oxoid™ Buffered Peptone Water (ISO)	CM1049B

Materials for lysis

Table 4 Materials for lysis of enriched cultures

Item	Source
Plastics, consumables, and reagents	
Single-channel pipette, 10- to 100- μ L <i>or</i> Electronic adjustable spacing, multichannel pipette, 10- to 100- μ L	Available through the Thermo Fisher Microbiology ordering process.
Single-channel stepper pipette, 10- to 100- μ L	
Filtered pipette tips, 10- to 100- μ L	
Filtered pipette tips for stepper pipette, 10- to 100- μ L	
Compact PCR tube rack, mixed colors	
Tool for capping and decapping	
Additional materials for the heat block method	
Heat block	MLS
Timer	
Additional materials for the thermal cycler method	
Applied Biosystems™ SimpliAmp™ Thermal Cycler	A24811
MicroAmp™ 96-Well Tray/Retainer Set for Veriti™ Systems ^[1]	4381850
MicroAmp™ Splash-Free 96-Well Base ^[1]	4312063

^[1] Included in the original instrument purchase.

Materials for PCR

Table 5 Materials for PCR

Item	Source
Real-time PCR instrument and accessories, one of the following instrument packages	
QuantStudio™ 5 Real-Time PCR Instrument, 0.1-mL block, with RapidFinder™ Analysis Software v1.1 or later For use with SureTect™ Escherichia coli O157:H7 PCR Assay and Pathogen Assay File: E.coliO157H7_SureTect_QS5 version 2.0 or later	A36320 (desktop) A36328 (laptop) Contact your local microbiology sales representative
7500 Fast Real-Time PCR Instrument with RapidFinder™ Express Software v2.0 or later For use with SureTect™ Escherichia coli O157:H7 PCR Assay and Pathogen Assay File: E.coliO157H7 SureTect 2.0 or later	A30304 (desktop) A30299 (laptop) Contact your local microbiology sales representative
Additional materials for PCR	
Vortex mixer	Available through the Thermo Fisher Microbiology ordering process. See thermofisher.com/plastics for more information.
8-channel pipette, 5- to 50-µL	
Filtered pipette tips, 10- to 100-µL	
For the QuantStudio™ 5 Real-Time PCR Instrument	
MicroAmp™ 96-Well Tray for VeriFlex™ Block	4379983
MicroAmp™ Splash-Free 96-Well Base	4312063
For the 7500 Fast Real-Time PCR Instrument	
Precision Plate holder for SureTect™ assays or 7500 Fast Precision Plate Holder, for 0.1 mL tube strips	PT0690 or A29252
PCR Carry plate for SureTect™ assays	PT0695
If using Precision Plate holder for SureTect™ assays (Cat. No. PT0690):	
Low Profile Tubes, strips of 8, white ^[1]	AB0771W
Ultra Clear qPCR Caps, strips of 8 ^[1]	AB0866
If using 7500 Fast Precision Plate Holder, for 0.1 mL tube strips (Cat. No. A29252):	

Item	Source
MicroAmp™ Fast 8-Tube Strip, 0.1 mL ^[2]	4358293
MicroAmp™ Optical 8-Cap Strips ^[2]	4323032

^[1] Used for balancing.

^[2] Required to balance the lid pressure if less than 2 full strips are processed.

Materials for confirmation testing

Table 6 Materials for confirmation of positive results

Item	Source
Oxoid™ Sorbitol MacConkey Agar	CM0813B
Oxoid™ C-T Supplement	SR0172E
Dynabeads™ anti-E. coli O157	71003
Dry-Bags™ Maximum Recovery Diluent (MRD)	DB0733W
Escherichia coli O157 Latex Test	DR0620M
Remel™ RIM E. coli O157:H7 Latex Test	R24250
Oxoid™ Novobiocin Selective Supplement	SR0181E, or equivalent

Validated workflows

Enrich food samples—raw beef meat and fruits and vegetables (page 13)



Prepare the lysate using the thermal cycler method (page 15)

or

Prepare the lysate using the heat block method (page 17)



“PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v1.1 or later” on page 18

Prepare the lysate using the thermal cycler method (page 15)

or

Prepare the lysate using the heat block method (page 17)



“PCR with the 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later” on page 20



Confirm positive results (page 23)



Before you begin

Procedural guidelines

Guidelines for sample enrichment

- For preparation of master suspensions, follow the instructions of EN ISO 6887 and EN ISO 16654 standards. Comply with Good Laboratory Practices (refer to EN ISO 7218 standard).
- Follow the manufacturer's instructions for preparation of culture media.
- The short protocols of detection are sensitive to conditions of incubation. It is required to respect scrupulously the conditions of temperature indicated in the technical specification. Notably, you must verify that the temperature or pre-warming of the enrichment broth allows to reach the temperature required. The time of preparation of the samples, delay between the end of the step of pre-warming of the enrichment broth and the beginning of the step of incubation of the food samples, does not exceed 45 minutes. Using a ventilated incubator during incubation is recommended.
- Use filtered homogenizer bags to help with fat and particle separation.
- For consistent PCR results, use a ventilated incubator.
- Follow the specified temperature allowances.
- Dispose of all inoculated culture media as hazardous microbiological waste, even if shown to be negative for the target organism, according to local guidelines.

Guidelines for sample lysis

- Follow the specified temperature allowances.
- For downstream PCR on the **7500 Fast instrument or the QuantStudio™ 5 Instrument** — Prepare a mock-purified sample using sterile enrichment media as a negative extraction control. (The negative extraction control is required for RapidFinder™ Express Software; it is optional but recommended for RapidFinder™ Analysis Software.)
Add the enriched sample or negative extraction control to the bottom of the lysis tube.
- For the **thermal cycler method** — To prevent crushing tubes, use the tray *only* from the MicroAmp™ 96-Well Tray/Retainer Set provided with the SimpliAmp™ Thermal Cycler. See the *SimpliAmp™ Thermal Cycler User Guide* (Pub. No. MAN0009889). Alternatively, use at least 4 complete tube strips in the heat block. We recommend spacing the strips evenly across the heat block. If needed, add empty SureTect™ tubes to make 4 complete strips.

Guidelines for PCR

- **IMPORTANT!** After the lysate has been added to the pellets, ensure that the pellet rehydrates immediately by tapping the tubes on the lab bench. Start the PCR run within 30 minutes.
- Tube and cap strips can be cut when less than a full strip is required. Do not cut the strips of caps or tubes too close to the wall of the tube or the cap lid, otherwise the lid might not seal adequately during PCR.
- After the PCR tubes have been opened, add lysate within 10 minutes.
- Particulate matter from the lysate can inhibit the PCR. To ensure that no particles are transferred from the Lysis Reagent 1 Tube to the PCR tube, remove lysate from the top half of the liquid, taking care not to disrupt the particles at the bottom of the tube.
If the particles become disturbed, allow the particles to resettle for 1–2 minutes before lysate removal.
- Ensure that the pellet is fully dissolved. The solution changes from blue to green when the pellet is dissolved.
- For ease of use, a multi-channel pipettor can be used to transfer multiple lysates to the PCR tubes.
- Follow “Good laboratory practices for PCR” on page 29. For more information go to www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html.



Figure 1 Avoid lysis particles

Enrich food samples—raw beef meat and fruits and vegetables

1. For the raw beef meat samples only, pre-warm the indicated volume of media to $41.5\pm 1^\circ\text{C}$.
2. Transfer the food sample to a homogenizer bag, then add the media, as indicated.

Table 7 Enrichment conditions

Matrices	PCR instrument	Media	Incubation
25 g raw beef meat	<ul style="list-style-type: none"> • QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v1.1 or later • 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later 	1-in-10 ratio of sample to media For example, 25 g of sample and 225 mL of pre-warmed Buffered Peptone Water (ISO)	$41.5\pm 1^\circ\text{C}$ for 8-24 hours
25 g fruits and vegetables <ul style="list-style-type: none"> • Sprouts • Leafy greens • Fruits and juices 		1-in-10 ratio of sample to media For example, 25 g of sample and 225 mL of Buffered Peptone Water (ISO)	$41.5\pm 1^\circ\text{C}$ for 20-24 hours

3. Homogenize the sample.
 - For soft samples—homogenize for 30 seconds to 1 minute using a homogenizer.
 - For samples containing hard particles, such as bone—squeeze the bag by hand until the sample is mixed thoroughly with the media.
4. Incubate as described in Table 7.
5. Remove the enriched sample from the incubator, briefly mix the liquid in the homogenizer bag/tube by hand, transfer an aliquot of sample from the filtered side of the bag to a new tube, then close the tube and briefly mix.
Retain sufficient sample for confirmation or repeat testing.

Proceed directly to Chapter 4, “Prepare the lysate”, or store the retained sample at 2–8°C for a maximum of 72 hours. Do not exceed 72 hours of total storage time.



Prepare the lysate

Prepare the lysate using the thermal cycler method

1. Equilibrate the Lysis Reagent 1 Tubes to room temperature.
 - a. Place the required number of Lysis Reagent 1 Tubes in a MicroAmp™ Splash-Free 96-Well Base and MicroAmp™ 96-Well Tray.
 - b. Check that there is no liquid around the plastic seal and the reagents are collected at the bottom of each tube.
 - c. Allow the tubes to remain at room temperature for approximately 10 minutes before opening.

2. Remove the plastic seal from each Lysis Reagent 1 Tube, then add 10 µL of Proteinase K to the tube.

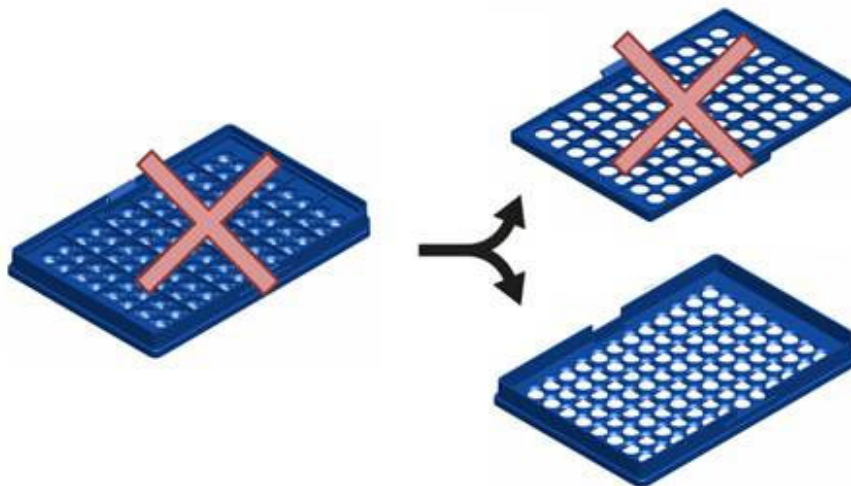
These tubes are referred to as Lysis Tubes in the rest of the procedure.

IMPORTANT! Avoid contamination of the Proteinase K stock tube. Use a new filtered pipette tip each time Proteinase K is withdrawn from the stock tube. Use a 10–100 µL repeat pipettor to reduce the number of tips required.

3. Transfer 10 µL of the enriched sample to a Lysis Tube. For the negative extraction controls, transfer 10 µL of sterile enrichment media to a Lysis Tube. Ensure that the pipette tip reaches the bottom of the Lysis Tube, to facilitate complete mixing of the sample with Lysis Reagent 1.

- Seal the tubes with the domed Lysis Tube Caps using the capping tool, then incubate the samples in the SimpliAmp™ Thermal Cycler using the following program.

IMPORTANT! To prevent crushing the tubes in the SimpliAmp™ Thermal Cycler, use the bottom piece from the MicroAmp™ 96-Well Tray/Retainer Set or include at least 4 complete SureTect™ Lysis tube strips (see “Guidelines for sample lysis” on page 11).



Ensure that the lid heater is on and set to 105°C, and the volume is set to Maximum.

Step	Temperature	Time
1	37°C	10 minutes
2	95°C	5 minutes
3	10°C	2 minutes
4	4°C	Hold ^[1]

^[1] For convenience, samples can be held at 4°C until proceeding to PCR or transfer to storage at 2–8°C.

- Proceed directly to Chapter 5, “Perform PCR”.

(Optional) Store the samples at 2–8°C for up to 24 hours, including any time stored at 4°C in the thermal cycler.

Prepare the lysate using the heat block method

1. Ensure that two heating blocks are set to $37\pm 2^{\circ}\text{C}$, and $95\pm 2^{\circ}\text{C}$.
2. Equilibrate the Lysis Reagent 1 Tubes to room temperature.
 - a. Place the required number of Lysis Reagent 1 Tubes in a MicroAmp™ Splash-Free 96-Well Base and MicroAmp™ 96-Well Tray.
 - b. Check that there is no liquid around the plastic seal and the reagents are collected at the bottom of each tube.
 - c. Allow the tubes to remain at room temperature for approximately 10 minutes before opening.

3. Remove the plastic seal from each Lysis Reagent 1 Tube, then add 10 μL of Proteinase K to the tube.

These tubes are referred to as Lysis Tubes in the rest of the procedure.

IMPORTANT! Avoid contamination of the Proteinase K stock tube. Use a new filtered pipette tip each time Proteinase K is withdrawn from the stock tube. Use a 10–100 μL repeat pipettor to reduce the number of tips required.

4. Transfer 10 μL of the enriched sample to a Lysis Tube. For the negative extraction controls, transfer 10 μL of sterile enrichment media to a Lysis Tube. Ensure that the pipette tip reaches the bottom of the Lysis Tube, to facilitate complete mixing of the sample with Lysis Reagent 1.
5. Seal the tubes with domed Lysis Tube Caps using the capping tool, then incubate the samples in the appropriate heating blocks:
 - a. $37\pm 2^{\circ}\text{C}$ for 10 minutes
 - b. $95\pm 2^{\circ}\text{C}$ for 5 minutes
 - c. Ambient temperature for 2 minutes
For convenience, samples can be transferred to storage at $2\text{--}8^{\circ}\text{C}$ for up to 24 hours.
6. Proceed directly to Chapter 5, “Perform PCR”.



Perform PCR

PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v1.1 or later

This method is validated for lysates that are prepared using either the thermal cycler or heat block methods.

Set up the plate layout in RapidFinder™ Analysis Software

The plate layout is determined by the user. See the Help function in the software for detailed instructions.

In the home screen of RapidFinder™ Analysis Software, click **Create Experiment**, then enter or edit the well parameters.

Select **E.coliO157H7_SureTect_QS5 version 2.0** or later for the assay.

Set up the PCR reactions

Before starting this procedure, ensure that you are familiar with “Guidelines for PCR” on page 12.

1. Following the plate layout previously set up in the software, place the required number of SureTect™ Escherichia coli O157:H7 PCR Tubes (PCR tubes) in the MicroAmp™ 96-Well Tray for VeriFlex™ Block. Place the block on the MicroAmp™ Splash-Free 96-Well Base. Press the PCR tubes to the tray to ensure they sit firmly, then tap the tubes on the bench to ensure that the pellets are located at the bottom of the tubes.
2. Allow the PCR tubes to remain on the bench for approximately 5 minutes, to bring to room temperature ($23\pm 5^{\circ}\text{C}$), then open one strip of PCR tubes by removing the seal.

IMPORTANT!

- If all sample lysates can be applied to the PCR tubes in 10 minutes, then open *all* strips of the PCR tubes.
 - If all sample lysates cannot be applied to the PCR tubes in 10 minutes, then open *only one* strip of the PCR tubes, then proceed to the next step.
-
- PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
 - If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty, pipette tip. Do not use a tip containing lysate.
3. Uncap the Lysis Tubes using the decapping tool.

- Transfer 20 µL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet.

IMPORTANT! Remove lysate from the top half of the liquid to ensure that no lysis particles are transferred from the Lysis Tube to the PCR tube. Do not touch the pellet when adding the lysate.

- Seal the PCR tubes with the flat optical PCR Caps provided with the kit. Ensure that the tubes are properly sealed by pressing down firmly over each opening. Do not use the capping tool to seal the PCR tubes.
- If *only one* strip of PCR tubes was opened, then repeat steps 2–5 for the remaining strips of PCR tubes.
- Mix all PCR tubes thoroughly for 10–15 seconds to ensure that the pellet is fully rehydrated.
 Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. If needed, hold the tubes upright, and flick sharply downward.

IMPORTANT! Start the PCR run within 30 minutes of addition of sample lysates to the PCR tubes.

Load and run the reactions

- Eject the instrument drawer. Use the MicroAmp™ 96-Well Tray for VeriFlex™ Block to transfer the tubes to the instrument in the same configuration as the plate layout determined in the software, then close the instrument drawer.
- In the **Run** tab of the experiment file in RapidFinder™ Analysis Software, select the instrument's serial number from the **Instrument** drop-down list.
- Click **Start Run**, then follow the software prompts.




View results and data analysis

Data analysis is automated by the software. For detailed instructions, and options for reporting, export, and storage of results, see the Help function in the software.

In the home screen of the RapidFinder™ Analysis Software, click **Results**, then click the sub-tab for the desired view of the data.

- Summary**—plate format
- Results**—table format
- Details**—amplification plot

RapidFinder™ Analysis Software results icons

Result icon	Result
	Positive result
	Negative result
	Result warning

PCR with the 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later

This method is validated for lysates that are prepared using either the thermal cycler or heat block methods.

Set up the plate layout

RapidFinder™ Express Software determines the Run Layout (plate layout) for your samples based on the information entered, and creates a run file. Refer to the Help function in the software for more details.

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

If desired, you can manually customize the plate layout in the software.

Select **E.coliO157H7 SureTect 2.0 or later** for the assay.

Set up the PCR reactions

Before starting this procedure, ensure that you are familiar with “Guidelines for PCR” on page 12.

1. Following the plate layout previously set up in the software, place the required number of SureTect™ Escherichia coli O157:H7 PCR Tubes (PCR tubes) in a suitable rack with a PCR carry plate, then tap the rack of tubes on the bench to ensure that the pellets are located at the bottom of the tubes.
If required by the plate layout, place empty low profile PCR tubes in the rack to balance the tray when the tubes are placed in the instrument.
2. Allow the PCR tubes to remain on the bench for approximately 5 minutes, to bring to room temperature ($23\pm 5^{\circ}\text{C}$), then open one strip of PCR tubes by removing the seal.

IMPORTANT!

- If all sample lysates can be applied to the PCR tubes in 10 minutes, then open *all* strips of the PCR tubes.
 - If all sample lysates cannot be applied to the PCR tubes in 10 minutes, then open *only one* strip of the PCR tubes, then proceed to the next step.
-
- PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
 - If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty, pipette tip. Do not use a tip containing lysate.
3. Uncap the Lysis Tubes using the decapping tool.


4. Transfer 20 µL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet.

IMPORTANT! Remove lysate from the top half of the liquid to ensure that no lysis particles are transferred from the Lysis Tube to the PCR tube. Do not touch the pellet when adding the lysate.

5. Seal the PCR tubes with the flat optical PCR Caps provided with the kit. Ensure that the tubes are properly sealed by pressing down firmly over each opening. Do not use the capping tool to seal the PCR tubes.
6. If *only one* strip of PCR tubes was opened, then repeat steps 2–5 for the remaining strips of PCR tubes.
7. Mix all PCR tubes thoroughly for 10–15 seconds to ensure that the pellet is fully rehydrated.
Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. If needed, hold the tubes upright, and flick sharply downward.

IMPORTANT! Start the PCR run within 30 minutes of addition of sample lysates to the PCR tubes.

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. Use the PCR carry plate to transfer the tubes to the instrument in the same configuration as the run layout.
Use the Precision Plate Holder for SureTect™ assays.
Be sure to load empty SureTect™ PCR tube strips as directed by the software (Figure 2).
2. Close the tray to the instrument, and follow the RapidFinder™ Express Software prompts to start the run.

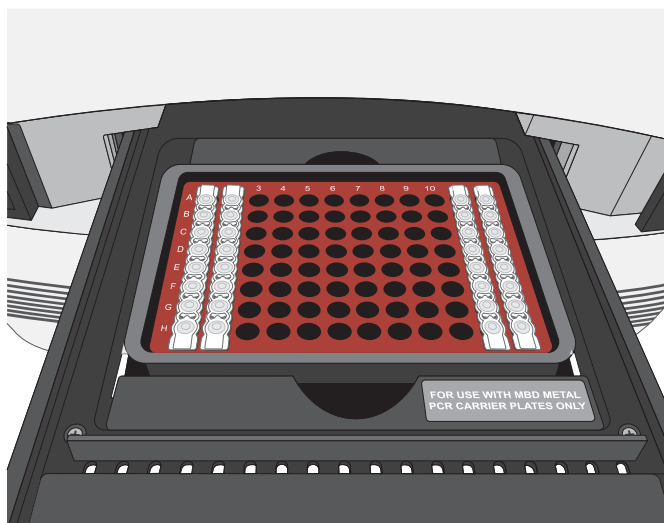



Figure 2 7500 Fast instrument tube layout

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 results and data analysis

Data analysis is automated by the software.

In the 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 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.

Options for reporting results

See the RapidFinder™ Express Software Help function for options for reporting, export, and storage of results.



Confirm positive results

Recommended confirmation methods

In the context of NF VALIDATION™ certification, samples with positive PCR results must be confirmed by one of the following tests.

- Perform selective plating and the latex test (see “Isolate presumptive positives” on page 23).
- Using conventional tests described in the methods standardized by CEN or ISO from colonies (including the purification step). The confirmation step must start from the primary enrichment broth.

In the event of discordant results (presumptive positive with the alternative method, not confirmed by one of the means described above/below and in particular by the Latex test), the laboratory must employ adequate means to ensure the validity of the result obtained.

Isolate presumptive positives

Positive results can be confirmed by immuno-concentration of 1 mL of the enrichment using Dynabeads™ anti-E. coli O157 magnetic beads.

1. Perform immunomagnetic separation according to the Dynabeads™ anti-E. coli O157 product instructions. Ensure that the final elution volume of the concentrated sample is 100 µL.
2. Pipet, then streak 50 µL of the beads onto Oxoid™ Sorbitol MacConkey Agar with Oxoid™ C-T Supplement (CT-SMAC agar), then incubate for 18–24 hours at 37±1°C.
3. Confirm well-isolated, presumptive positive *Escherichia coli* colonies using one or more of the following:
 - The *Escherichia coli* O157 Latex Test
 - Remel™ RIM E. coli O157:H7 Latex Test—When confirming for the H7 antigen using Remel™ RIM E. coli O157:H7 Latex Test, subculture suspect colonies from the CT-SMAC agar onto blood agar.
 - The ISO horizontal method for the detection of *Escherichia coli* O157:H7 (EN ISO 16654-2001)—During enrichment, supplement the mTSB with 20 mg/L Oxoid™ Novobiocin Selective Supplement, and take a sample of the enrichment media after 6 hours and 18–24 hours of incubation.
 - The USDA FSIS MLG reference methods



Troubleshooting

Observation	Possible cause	Recommended action
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"> 1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
In negative extraction control wells, no IPC signal is detected, but a target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred. Additionally, a problem with the IPC occurred due to: <ul style="list-style-type: none"> • Preferential amplification of the carryover DNA. • Carryover of particles from the Lysis Tube. 	<ol style="list-style-type: none"> 1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
In negative extraction control wells, no IPC signal or an exceptionally weak or atypical IPC amplification plot is detected. The result is considered invalid by the software.	Pellets are not fully dissolved before starting the PCR run.	Mix thoroughly for 10-15 seconds to ensure the pellet is fully rehydrated.
	Incomplete lysis steps cause an inhibition of the PCR.	Retest the original sample and its dilution, ensuring that the correct heating parameters are followed.
In test samples, no IPC nor target-specific signal is detected, and/or an exceptionally weak or atypical amplification plot is observed. The result is considered invalid by the software.	Inhibition of PCR occurred, due to: <ul style="list-style-type: none"> • Carryover of particles from the Lysis Tube. • PCR inhibitors present in the food sample. • Other, unknown, cause. 	Retest the original sample and its dilution. To remove the impact of PCR inhibitors in the sample, dilute the enriched sample 1-in-5 (1 part enriched sample and 4 parts sterile media), or 1-in-10 (1 part enriched sample and 9 parts sterile media), then repeat the sample lysis procedure and PCR.
	Pellets are not fully dissolved before starting the PCR run.	Mix thoroughly for 10–15 seconds to ensure the pellet is fully rehydrated.
	Inhibition of PCR caused by incomplete lysis steps.	Retest the original sample and its dilution. To remove the impact of PCR inhibitors in the sample, dilute the enriched sample 1-in-5 (1 part enriched sample and 4 parts sterile media), or 1-in-10 (1 part enriched sample and 9 parts sterile media), then repeat the sample lysis procedure and PCR.



Observation	Possible cause	Recommended action
In test samples, no IPC signal is detected, but target-specific signal is detected. The result is considered invalid by the software.	A problem occurred in IPC amplification due to preferential amplification of the target-specific DNA.	Retest the original sample and its dilution. Dilute the enriched sample 1-in-5 (1 part enriched sample and 4 parts sterile media) or 1-in-10 (1 part enriched sample and 9 parts sterile media), then repeat the sample lysis procedure and PCR.
In test samples that are expected to be positive, no target-specific signal is detected	Certain sample types may contain components that are inhibitory to the growth of the target organism.	Pre-treat or dilute the sample after enrichment. See the appropriate local, national or international guidelines.
In confirmation testing, suspect colonies on CT-SMAC Agar are not present	Overgrowth of <i>E. coli</i> O157 by background flora, or the IMS capture of <i>E. coli</i> O157:H7 failed.	Perform a dilution series from the IMS bead elution plate onto the CT-SMAC Agar.
		Streak the retained sample directly onto the CT-SMAC Agar.
In confirmation testing, suspect colonies on CT-SMAC Agar are too small to conduct confirmation tests	The isolate is sensitive to selective components in the medium or the lower limit of the incubation time was used.	Purify the well-isolated, suspect colony on a non-selective plating medium to increase biomass before continuing with confirmation.
		Perform a dilution series from the IMS bead elution plate by spreading 50 µL onto the CT-SMAC agar.
In confirmation testing, suspect colonies on CT-SMAC Agar are not well isolated	The enriched sample contains high levels of background flora that were not inhibited on CT-SMAC Agar.	Purify the suspect colonies on a second CT-SMAC plate.

Test control organisms

Incorporation of positive control organisms is not necessary with routine testing of samples, because the PCR results are validated if the IPC signal is detected.

If testing of positive control organisms is required, select suitable organisms. Quality control organisms are available from Thermo Fisher Scientific, Microbiology Division. Contact your local supplier for further information.

Process a control organism in parallel with test samples through sample enrichment, lysis, and PCR, following your laboratory methodology.



RapidFinder™ Express Software results warnings

RapidFinder™ Express Software v2.0 may indicate a result warning due to inhibition for some samples. In some rare cases the warning label is result of **Non-linear baseline** notification for the bacterial targets and/or IPC detector of the assay.

In such rare cases, follow the recommended workflow:

1. Select **View details** to manually view results of the highlighted reaction for the bacterial targets and the IPC in the RapidFinder™ Express Software v2.0.
2. Inspect the IPC result.
3. Inspect the bacterial target results.

If the C_t of the IPC is below the cut off C_t value depicted in following table and the bacterial targets have received a negative interpretation and the signal is above the cut off C_t value, the result can be interpreted as true negative.

Whenever the IPC and bacterial targets have received C_t values below the cut off C_t values depicted in the following table , proceed to a confirmation step as described in the user guide.

In case of a negative IPC result or IPC C_t above the cut off, follow the instructions given in the user guide to repeat the sample.

Assay	Cut off for target C_t value	Cut off for IPC C_t value
SureTect™ Escherichia coli O157:H7 PCR Assay	50 (both targets)	40



Supplemental information

NF VALIDATION™ by AFNOR certification

Go to thermofisher.com/foodsafety for a guide to workflows for detection of *Escherichia coli* O157:H7 (Pub. No. MAN0009419).

Table 8 NF Validation certification of the workflow

Certification	Expiration
 <p>UNI 03/10 - 03/15 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS http://nf-validation.afnor.org/en</p>	See the certificate available at nf-validation.afnor.org/en or thermofisher.com/foodsafety .

The SureTect™ *Escherichia coli* O157:H7 PCR Assay has been certified “NF VALIDATION™”. The certification uses the ISO 16140 standard for the validation of alternative methods (Alternative Analytical Methods for Agribusiness. Certified NF VALIDATION™; nf-validation.afnor.org/en). This kit was compared and found equivalent to the ISO 6579 reference method. The validated workflow described in this user guide includes:

- Enrichment as described in “Enrich food samples—raw beef meat and fruits and vegetables” on page 13
- SureTect™ *Escherichia coli* O157:H7 PCR Assay

- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 9 for characteristics) with RapidFinder™ Analysis Software v1.1 or later and Pathogen Assay File: **E.coliO157H7_SureTect_QS5 version 2.0** or later

Table 9 QuantStudio™ 5 Real-Time PCR Instrument characteristics

Characteristics	QuantStudio™ 5 Real-Time PCR Instrument
Optics	Bright white LED
Filters	6 excitation and 6 emission filters
Sample ramp rate	Average: 3.66°C/sec Maximum: 9.0°C/sec
Thermal range	4-99°C
Thermal accuracy	±0.25°C
Thermal uniformity	±0.4°C
Format	96-well, 0.1-mL block

- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 10 for characteristics) with RapidFinder™ Express Software v2.0 or later, and Pathogen Assay File: **E.coliO157H7 SureTect 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: ±1.6°C/sec Fast mode: ±3.5°C/sec
Thermal range	4-100°C
Thermal accuracy	±0.5°C
Thermal uniformity	±1°C
Format	96-well, 0.1-mL block

- Confirmation testing of positive samples, as described in “Recommended confirmation methods” on page 23

Table 11 Validated matrices

Matrices ^[1]
25 g raw beef meet products 25 g fruits and vegetables <ul style="list-style-type: none"> • Sprouts • Leafy greens and frozen vegetables • Fruits and juices

^[1] Validated using the EN ISO 16654 method.

Good laboratory practices for PCR







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).

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.

Symbol definitions

Symbol	Definition
LOT	BATCH CODE

Symbol	Definition
	CATALOG NUMBER
	CONTAINS SUFFICIENT FOR <n> TESTS
	CONSULT INSTRUCTIONS FOR USE
	MANUFACTURER
	UPPER AND LOWER TEMPERATURE LIMIT (storage temperature)
	USE BY



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, 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 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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.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

Food Safety support

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

Support email:

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

Phone: Visit thermofisher.com/support, select the link for phone support, and select the appropriate country from the dropdown menu.

Related documentation

All of the SureTect™ IFUs are located at www.thermofisher.com/suretect-ifu.

Document	Publication number
<i>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i>	MAN0010407
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide</i>	4378657
<i>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide</i>	4387777
<i>SimpliAmp™ Thermal Cycler User Guide</i>	MAN0009889
<i>SimpliAmp™ Thermal Cycler Installation and Operation Quick Reference</i>	A24827
<i>RapidFinder™ Express Software Quick Reference</i>	4480999
<i>PCR Starter Kit for 96-well blocks, 0.2 mL, User Guide</i>	A24829

References

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

EN ISO 6887-1:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions.

EN ISO 6887-2:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 2: Specific rules for the preparation of meat and meat products.

EN ISO 6887-3:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 3: Specific rules for the preparation of fish and fishery products.

EN ISO 6887-4:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 4: Specific rules for the preparation of miscellaneous products.

EN ISO 6887-5:2010. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 5: Specific rules for the preparation of milk and milk products.

EN ISO 7218:2007. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.

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 definition.

