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SureTect™ Listeria monocytogenes PCR Assay

USER GUIDE

Lysis and real-time PCR detection of *Listeria monocytogenes* in food and environmental samples

for use with:

Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument with Thermo Scientific™ RapidFinder™ Analysis Software v2.0 or later

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

Catalog Number PT0300A

Publication Number MAN0017806

Revision D.0

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 061302

Official Methods of Analysis of AOAC INTERNATIONAL

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For testing of Food and Environmental samples only.

ThermoFisher SCIENTIFIC

Revision history: Pub. No. MAN0017806

Revision	Date	Description
D.0	6 July 2023	• New SKUs were added for the Oxoid™ 24 LEB Buffer Supplement. • (ISO) designation was added for Oxoid™ *Brilliance*™ products. • A note was added for instrument dye calibration. • The RapidFinder™ Analysis Software version was updated. • The RFE assay file name was updated.
C.0	8 December 2020	• The software versions were updated. • The workflow was clarified to indicate either type of PCR instrument can be used regardless of how lysate is prepped.
B.0	16 October 2019	• References to Boekel Scientific heating blocks were removed. • The pathogen assay file names were updated. Composite foods and environmental samples matrices were added.
A.0	12 December 2018	New document for SureTect™ Listeria monocytogenes PCR Assay with new publication number. Supersedes Pub. No. D12105 Version 10. Additional changes: • NF VALIDATION™ by AFNOR Certification workflow that covers enrichment, DNA isolation, and real-time PCR detection was added. • Instructions for PCR on the QuantStudio™ 5 Instrument were added. • Matrices were updated from only all human foods to include meat products, milk and dairy products, seafood and fishery products, vegetables, and environmental samples. • Windows™ 10 software support was added. • Assay kit file references were corrected. • A mixing step to ensure pellet is rehydrated was added in "Set up the PCR reactions." • PikoReal™ Real-Time PCR System was removed following equivalence studies approved by AFNOR Certification and AOAC INTERNATIONAL with the 7500 Fast Real-Time PCR System and QuantStudio™ 5 Real-Time PCR System. • The document was updated to the current template with associated changes in document organization, licensing, trademarks, and logos.

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

Contents

1 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™ Listeria monocytogenes PCR Assay enables real-time PCR detection of *Listeria monocytogenes* from food and environmental samples. This kit is for use in laboratories undertaking microbiological analysis. See Table 1 for compatible instruments and software.

Note: The qPCR instrument must be calibrated with the following dyes before use: JUN™ and Cy5™.

Table 1 Instruments and software

PCR instrument	Software	Pathogen Assay File
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument	Thermo Scientific™ RapidFinder™ Analysis Software v2.0 or later	**ListeriaMono_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	**Listeria_monocytogenes_SureTect_7500_2.0** or later[1]

[1] Assay files and instructions are available at **thermofisher.com/molecular-microbiology-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 *Listeria monocytogenes* 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/qpcreducation**.

The assay includes an internal positive control for each reaction to confirm that the PCR process has occurred. It is unnecessary to incorporate positive control organisms with routine testing of samples.

Procedure overview

Enriched food and environmental samples are combined directly with ready-to-use Lysis Reagent 1, Lysis Reagent 2, and Proteinase K to lyse bacterial cells present in the sample and release their DNA into solution.

Lysates are transferred to the SureTect™ Listeria monocytogenes 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 31 and EN ISO 22174:2005.
- For more information, see Appendix A, “Troubleshooting”.

Contents and storage

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

Table 2 SureTect™ Listeria monocytogenes PCR Assay, 96 tests (Cat. No. PT0300A)

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
Lysis Reagent 2 (clear colorless liquid)	1 tube
SureTect™ Listeria monocytogenes 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 3 Equipment, accessories, and consumables

Item	Source
Homogenizer laboratory blender or diluter, one of the following or equivalent:	
• Homogenizer Laboratory Blender • Diluflux™ Pro Automated Gravimetric Dilutor with simple (non-robotic) dispensing arm • Diluflux™ Pro Automated Gravimetric Dilutor with robotic dispensing arm	DB5000A DB4100A DB4150A
Sample enrichment bags, one of the following or equivalent:	
• BagFilter™ 400 (400 mL) • BagPage™ 400 (400 mL) • BagLight™ 400 (400 mL) • RollBag™ 1300 (1300 mL)	DB4011A DB4012A DB4013A DB4014A
Incubator fitted with racks for homogenizer bags	**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 4 Media

Item	Source
Oxoid™ 24 Listeria Enrichment Broth (24 LEB), Dehydrated culture powder	CM1107B (base 500 g)
FitBag™ 24 Listeria Enrichment Broth, Dehydrated culture media • 30 x 2.7 L • 20 x 4.5 L • 10 x 9 L	DF1107A DF1107B DF1107C
QuickBag™ 24 Listeria Enrichment Broth, Dehydrated culture media • 3 x 2.7 L • 2 x 4.5 L	DQ1107A DQ1107B
Dry-Bags™ 24 Listeria Enrichment Broth, Dehydrated culture media, (5 x 20 L)	DB1107V
Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Selective Supplement	SR0243E, 10X, 5 mL/bottle
Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement[1]	BO1339E, 24 × 10 mL BO1204M, 10 × 100 mL Available through the Thermo Fisher Microbiology ordering process.

[1] This product may crystallize during storage. If crystals are present, place the tube in a 37°C water bath for 5–10 minutes, or until all of the crystals are dissolved.

Table 5 Additional materials for enrichment of production environment samples

Item	Source
Dey-Engley Broth or other neutralizing broth, or Peptone Water, as appropriate for the sample type	MLS
Sterile sampling swabs or sponges, for example: Remel™ bio-spo Sponge	• Sponges: R658003 or equivalent • Swabs: MLS

Materials for lysis

Table 6 Materials for lysis of enriched cultures

Item	Source
Plastics, consumables, and reagents	
Single-channel pipette, 10- to 100-µL *or* Electronic adjustable spacing, multichannel pipette, 10- to 100-µL	Available through the Thermo Fisher Microbiology ordering process.
Single-channel stepper pipette, 10- to 100-µL	
Filtered pipette tips, 10- to 100-µL	
Filtered pipette tips for stepper pipette, 10- to 100-µL	
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 7 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 v2.0 or later For use with SureTect™ Listeria monocytogenes PCR Assay and Pathogen Assay File: **ListeriaMono_SureTect_QS5** version 2.0 or later	A36320 (desktop) A36328 (laptop) Contact your local microbiology sales representative

Table 7 Materials for PCR *(continued)*

Item	Source
7500 Fast Real-Time PCR Instrument with RapidFinder™ Express Software v2.0 or later For use with SureTect™ Listeria monocytogenes PCR Assay and Pathogen Assay File: **Listeria_monocytogenes_SureTect_7500_2.0** or later	A30304 (desktop) A30299 (laptop) Contact your local microbiology sales representative
For the QuantStudio™ 5 Food Safety Real-Time PCR Instrument	
MicroAmp™ 96-Well Tray for VeriFlex™ Block	4379983
MicroAmp™ Splash-Free 96-Well Base	4312063
For the 7500 Fast Food Safety Real-Time PCR Instrument	
PCR Carry plate for SureTect™ assays	PT0695
Plate holder, one of the following (with accessories): • Precision Plate holder for SureTect™ assays • 7500 Fast Precision Plate Holder, for 0.1 mL tube strips	PT0690 A29252
Accessories required for the Precision Plate holder for SureTect™ assays (Cat. No. PT0690): • Low Profile Tubes, strips of 8, white[1] • Ultra Clear qPCR Caps, strips of 8	AB0771W AB0866
Accessories required for the 7500 Fast Precision Plate Holder, for 0.1 mL tube strips (Cat. No. A29252): • MicroAmp™ Fast 8-Tube Strip, 0.1 mL[2] • MicroAmp™ Optical 8-Cap Strips	4358293 4323032
Additional materials for PCR	
Vortex mixer	Available through the Thermo Fisher Microbiology ordering process. See **thermofisher.com/plastics** for more information.
8-channel pipette, 10- to 100-µL	
Filtered pipette tips, 10- to 100-µL	

[1] Used for balancing.

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

Materials for confirmation testing

Table 8 Materials for confirmation of positive results

Item	Source
Oxoid™ *Brilliance*™ Listeria Agar (ISO) Base	CM1080B (base, 500 g)
Oxoid™ *Brilliance*™ Listeria Agar (ISO) Selective Supplement	SR0227E
Oxoid™ *Brilliance*™ Listeria Agar (ISO) Differential Supplement	SR0228E
Oxoid™ *Brilliance*™ Listeria Agar (ISO) (ISO) Base	CM1212B (base, 500 g)
Oxoid™ *Brilliance*™ Listeria Agar (ISO) Selective Supplement	SR0257E
Oxoid™ *Brilliance*™ Listeria Agar (ISO) Differential Supplement	SR0258E

Validated workflows

Enrich a broad range of food samples (page 15)

or

Enrich production environment samples (page 17)

▼

Prepare the lysate using the thermal cycler method (page 19)

▼

or

Prepare the lysate using the heat block method (page 21)

▼

PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v2.0 or later (page 22)

or

PCR with the 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later (page 25)

▼

Confirm positive results (page 28)

2 Before you begin

Procedural guidelines

Guidelines for sample enrichment

- For preparation of initial suspensions, follow the instructions of EN ISO 11290 and ISO 18593:2018 standards. Samples can be prepared according to EN ISO 6887 technical rules. Comply with Good Laboratory Practices (refer to EN ISO 7218:2007 standard).
- Follow the manufacturer's instructions for preparation of culture media.
- 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.

CAUTION! Pregnant women and immune-compromised individuals are advised not to carry out this test as it involves the possible culturing of *Listeria monocytogenes* or *Listeria* spp. which can result in harmful infections if not handled correctly. Please refer to your local laboratory procedures and safety requirements.

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.

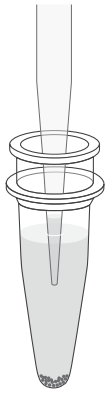


Figure 1 Avoid lysis particles

- Follow "Good laboratory practices for PCR" on page 36. For more information go to **www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html**.

Guidelines for spinning of PCR tubes

- SureTect™ workflow:
  - 20 µL of SureTect™ lysate is added to each PCR tube.
  - The PCR tubes are vortexed for 10–15 seconds to ensure that the pellet is fully rehydrated.
  - User must ensure that the reaction mixture is at the bottom of the PCR tube.
- A rapid spin-down is highly recommended before the PCR run to:
  - Collect the reaction mixture at the bottom of the well.
  - Remove bubbles.
- This ensures that the reaction conditions are optimal, and as a result, the PCR step is less likely to fail or to suffer from unwanted signal fluctuations which could affect the interpretation.
- Centrifugation of PCR tubes is included in every GLP (Good Laboratory Practice) protocol.

3 Enrich food or environmental samples

Enrich a broad range of food samples

1. Prepare Oxoid™ 24 LEB according to the manufacturer's instructions.
2. Prepare the media by combining:
   - 1 L of Oxoid™ 24 LEB
   - 10 mL (2 vials) of reconstituted Oxoid™ 24 LEB Selective Supplement
3. Pre-warm the media to room temperature (23±5°C).
4. Transfer the food sample to a homogenizer bag, then add room temperature (23±5°C) media, as indicated. See Table 9.

Table 9 Enrichment conditions for a broad range of foods

Matrices	PCR Instrument	Media	Incubation
Standard protocol for a Broad Range of Foods			
25 g • Milk and dairy products: Pasteurized 2% milk, Pasteurized brie cheese, Ice cream, Processed cheese, Blue cheese, Cottage cheese, Greek yogurt • Raw meat and raw poultry meat: Raw ground beef, Raw ground pork, Raw pork sausages, and Raw ground turkey • Ready-to-eat and Ready-to-reheat meat and poultry meat: Pork frankfurters, Cooked sliced ham, Salami, Cooked sliced turkey • Produces & fruits: Bagged lettuce, Cantaloupe melon, Fresh bagged spinach • Fish and seafood: Raw cod, Cooked prawns, Smoked salmon	• 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 prepared in step 2[1]	37±1°C for 22–30 hours
25 g Deli salad (Piemontaise), Pork rillettes, Raw milk, Smoked salmon, and Ready-to-cook vegetables			37±1°C for 20–28 hours
Specific protocol			
25 g Salami	• QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v1.1 or later • 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later	1-in-20 ratio of sample to media in step 2[2]	37±1°C for 22–30 hours

[1] For example, 25 g of sample in 225 mL of media.
[2] For example, 25 g of sample in 475 mL of media.

5. Add 10 mL (or 20 mL for Salami) of Oxoid™ 24 LEB Buffer Supplement per 25 g of sample, to the media in the homogenizer bag.

6. 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.
7. Incubate as described in Table 9.
8. 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.

Enrich environmental samples

Obtain production environment samples

1. Pre-moisten a sterile sampling swab or sponge.
   - For sampling of areas that have been cleaned or treated with disinfectants and other cleaning agents, use a neutralizing broth, such as Dey-Engley Broth.
   - For other areas, use sterile Peptone Water or other equivalent diluent.
2. Rub the swab or sponge in both a horizontal and vertical direction across the entire sampling area.
3. Place the sample in the original packaging or other material that is suitable for transport.

   Samples may be held for up to 2 hours at room temperature (23±5°C) or 8 hours in the refrigerator prior to adding the samples to media (see "Enrich production environment samples" on page 17).

Enrich production environment samples

1. Prepare Oxoid™ 24 LEB according to the manufacturer's instructions.
2. Prepare the media by combining:
   - 1 L of Oxoid™ 24 LEB
   - 10 mL (2 vials) of reconstituted Oxoid™ 24 LEB Selective Supplement

3. Add samples to media, as indicated.

Table 10 Environmental sample enrichment conditions

Sample type	PCR Instrument	Volume of media/sample	Media	Incubation
Swabs	• QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v2.0 or later • 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later	10 mL	Prepared in step 2, and at room temperature	37±1°C for 22–30 hours
Sponges, plastic, stainless steel, ceramic, and concrete matrices		100 mL		
25 mL Process water		225 mL		37±1°C for 20–28 hours

4. Add 4.4 mL of reconstituted Oxoid™ 24 LEB Buffer Supplement per 100 mL of media, to the media in the homogenizer bag.

5. Homogenize thoroughly.

6. Incubate as described in Table 10.

7. Remove the enriched sample from the incubator, mix gently, then transfer an aliquot of sample to a new tube, and close the tube.

   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.

4 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.
3. Add 10 µL of Lysis Reagent 2 to the tube.

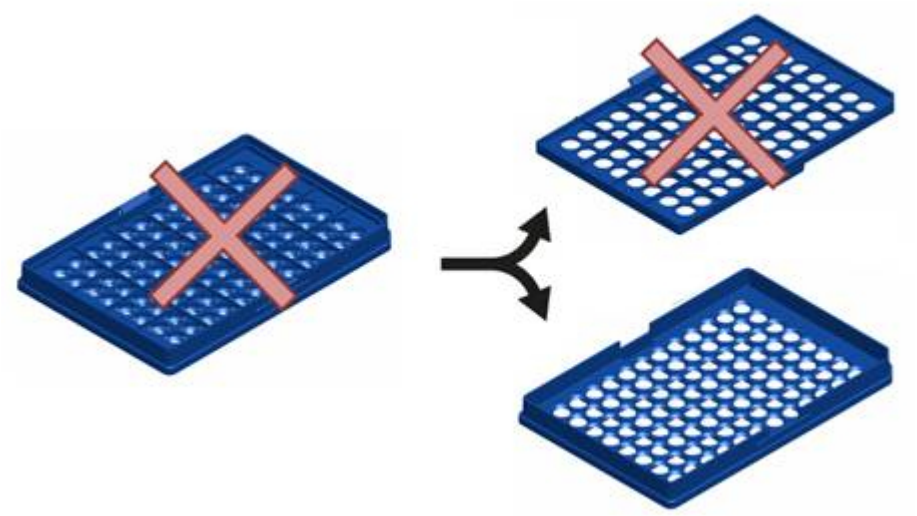
   These tubes are referred to as Lysis Tubes in the rest of the procedure.

   IMPORTANT! Avoid contamination of the Proteinase K or Lysis Reagent 2 stock tubes. Use a new filtered pipette tip each time Proteinase K or Lysis Reagent 2 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 the lysis reagents.

5. 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 13).



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.

6. Proceed directly to 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±2°C, and 95±2°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.
4. Add 10 µL of Lysis Reagent 2 to the tube.

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

   IMPORTANT! Avoid contamination of the Proteinase K or Lysis Reagent 2 stock tubes. Use a new filtered pipette tip each time Proteinase K or Lysis Reagent 2 is withdrawn from the stock tube. Use a 10–100 µL repeat pipettor to reduce the number of tips required.
5. 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 the lysis reagents.
6. Seal the tubes with domed Lysis Tube Caps using the capping tool, then incubate the samples in the appropriate heating blocks:
   a. 37±2°C for 10 minutes
   b. 95±2°C for 5 minutes
   c. Ambient temperature for 2 minutes

      For convenience, samples can be transferred to storage at 2–8°C for up to 24 hours.
7. Proceed directly to PCR.

5 Perform PCR

PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v2.0 or later

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 **ListeriaMono_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 14.

1. Following the plate layout previously set up in the software, place the required number of SureTect™ Listeria monocytogenes 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±5°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

1. 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.

2. In the **Run** tab of the experiment file in RapidFinder™ Analysis Software, select the instrument's serial number from the **Instrument** drop-down list.

3. 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

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 **Listeria_monocytogenes_SureTect_7500_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 14.

1. Following the plate layout previously set up in the software, place the required number of SureTect™ Listeria monocytogenes 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±5°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.

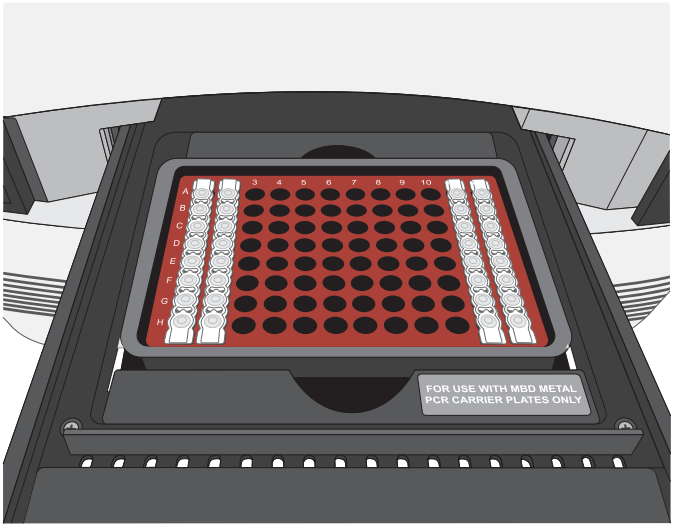


Figure 2 7500 Fast instrument tube layout

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.

6 Confirm positive results

Recommended confirmation methods

CAUTION! Pregnant women and immune-compromised individuals are advised not to carry out this test as it involves the possible culturing of *Listeria monocytogenes* or *Listeria* spp. which can result in harmful infections if not handled correctly. Please refer to your local laboratory procedures and safety requirements.

In the context of AOAC *Performance Tested Methods*℠ certification, samples with presumptive PCR positive results should be tested further using one of the following methods:

- Perform selective plating and the latex test (see "Isolate presumptive positives" on page 28).
- The appropriate reference method for the sample matrix (see "AOAC *Performance Tested Methods*℠ and AOAC First Action *Official Methods*℠ Certification" on page 33).

In the event of a positive PCR result, which cannot be confirmed using the steps described above, all necessary measures must be taken by the laboratory to establish the true status of the sample before reporting the result.

Isolate presumptive positives

1. Streak 10 µL from the stored enriched sample onto *Brilliance*™ Listeria Agar (ISO), then incubate for 22–26 hours at 37±1°C.

   If no colonies are present after 24 hours, re-incubate for another 24 hours.

2. Confirm well-isolated, presumptive positive *Listeria* colonies using the Oxoid™ Microbact™ Listeria 12L Kit, or the Oxoid™ Microbact™ Listeria 12L Haemolysin Reagent, or the confirmatory tests detailed in the ISO horizontal method for the detection of *Listeria* (EN ISO 11290).

   Note: Presumptive colonies for *Listeria monocytogenes* are blue with a halo.

7 Confirm presumptive colonies using SureTect™ Listeria monocytogenes PCR Assay - EN ISO 7218:2007

Confirm characteristic colonies using SureTect™ Listeria monocytogenes PCR Assay

The ISO 7218:2007 standard outlines the use of nucleic acid probes to confirm presumptive characteristic colonies if validation data is available [e.g. bibliography, ISO 16140, and/or AOAC validation study(ies)]. Therefore, the SureTect™ Listeria monocytogenes PCR Assay can be used for presumptive colony confirmation.

However, good laboratory practice suggests using different methods to screen and confirm samples. Adhering to this guidance, the SureTect™ Listeria monocytogenes PCR Assay should be used either for detection purposes or confirmation of characteristic colonies recovered on selective agar plates.

When using the SureTect™ Listeria monocytogenes PCR Assay to confirm colonies, the sample preparation is as follows:

1. Emulsify an isolated colony in 1 mL of saline.
2. Use this as a test sample by adding 10 µL input and follow the protocol described in Chapter 4, “Prepare the lysate”.
3. Perform PCR as described in Chapter 5, “Perform PCR”.

Note: It is recommended that users perform implementation verification as described in ISO 16140-3:2021.

A 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.	1. Repeat the assay using fresh aliquots of all reagents, fresh enrichment, 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: • Preferential amplification of the carryover DNA. • Carryover of particles from the Lysis Tube.	1. Repeat the assay using fresh aliquots of all reagents, fresh enrichment, 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 were not fully dissolved and/or lysate was not at the bottom of the tube before the PCR run was started.	Mix thoroughly by vortexing for 10–15 seconds to ensure the pellet is fully rehydrated and/or lysate is at the bottom of the tube.
	Incomplete lysis steps caused an inhibition of the PCR.	Retest the original sample and diluted sample, 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: • Carryover of particles from the Lysis Tube. • PCR inhibitors present in the food sample. • Incomplete sample lysis. • Other, unknown, cause.	Retest the original sample and its dilution. To remove the impact of PCR inhibitors in the sample, dilute the enriched sample 1:5 (1 part enriched sample and 4 parts sterile media), or 1:10 (1 part enriched sample and 9 parts sterile media), then repeat the sample lysis procedure and PCR.
	Pellets were not fully dissolved and/or lysate was not at the bottom of the tube before the PCR run was started.	Mix thoroughly by vortexing for 10–15 seconds to ensure the pellet is fully rehydrated and/or lysate is at the bottom of the tube.

Observation	Possible cause	Recommended action
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. *(continued)*	Bubbles were present in the PCR tube.	Inspect each tube for bubbles by looking through the optical PCR Caps. Large bubbles can often be removed by firmly holding the top of the tube while gently flicking the bottom. If the bubble persists, spin the tube for 10 seconds in a plate spinner. If the bubble continues to persist, set up a new PCR tube using the prepared lysate.
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 diluted sample. Dilute the enriched sample 1:5 (1 part enriched sample and 4 parts sterile media) or 1:10 (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 contained components that were inhibitory to the growth of the target organism.	Dilute the sample pre-enrichment to a level that prevents growth inhibition of target bacteria. For example, following guidelines in ISO 6887.
In confirmation testing, suspect colonies on *Brilliance*™ Listeria Agar (ISO) are not present	An overgrowth of *Listeria* with background flora occurred, or concentration of *Listeria* in the enrichment neared the detection limit of the assay.	Sub-culture 100 µL of the retained enrichment into 10 mL of suitable selective *Listeria* enrichment broth (24 LEB or Fraser Broth). Incubate at 37.0 °C for 24–28 hours before continuing with confirmation.
In confirmation testing, suspect colonies on *Brilliance*™ Listeria Agar (ISO) are too small to conduct a confirmation test	The isolate was 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.
In confirmation testing, suspect colonies on *Brilliance*™ Listeria Agar (ISO) are not well isolated	The enriched sample contained high levels of background flora that were not inhibited on *Brilliance*™ Listeria Agar (ISO).	Purify the suspect colonies on a second *Brilliance*™ Listeria Agar (ISO).

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. However, you may choose to use target isolates to ensure that the workflow, an assay, and/or a batch performs as it should.

If testing of positive control organisms is required, select a suitable organism recommended by 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.

The following instructions were generated to mitigate the risk of laboratory cross-contamination when handling target strains and provide general guidelines for positive control preparation.

IMPORTANT! Aseptic techniques and sterile consumables should be used at all the times.

- Strain selection and culture:
  - Select a suitable organism.
  - Streak the isolate onto an agar plate to obtain isolated colonies.
  - Incubate the plate under suitable conditions until colonies are visible easily by the naked eye.
- Sample preparation:
  - Pick a single well-isolated colony using a suitable sterile tool (e.g., pipette tip or culture loop).
  - Emulsify the colony carefully in 1 mL of saline or sterile enrichment broth.
  - Dilute the initial suspension using the same medium to obtain C_t value ~ 25-30 (e.g. 1:10 or 1:50).
  - Prepare lysate using 10 µL of diluted suspension instead of enriched sample. When possible, it is recommended to add the positive sample lysate on the PCR plate only after the (unknown) sample tubes have already been sealed.
  - Run PCR according to standard procedure.

    Note: Do not open the PCR tubes after the PCR run has completed.

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 the 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 target and the IPC in the RapidFinder™ Express Software v2.0.
2. Inspect the IPC result.
3. Inspect the bacterial target result.

If the C_t of the IPC is below the cut off C_t value depicted in following table and the bacterial target 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 target 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™ Listeria monocytogenes PCR Assay	50	40

B

Supplemental information

AOAC *Performance Tested Methods*℠ and AOAC First Action *Official Methods*℠ Certification

Table 11 *Performance Tested Methods*℠ and *Official Methods*℠ Certification of the workflow

Performance Tested Methods℠ Certification	*Official Methods*℠ Certification
PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 061302	Official Methods of Analysis of AOAC INTERNATIONAL First Action 2021.05 AOAC First Action *Official Methods*℠ (No. 2021.05) https://www.aoac.org/scientific-solutions/standards-and-official-methods/

The detection of *Listeria monocytogenes*, using the SureTect™ Listeria monocytogenes PCR Assay has earned the AOAC *Performance Tested Methods*℠ Certification and the AOAC First Action *Official Methods*℠ Certification from the AOAC Research Institute. The certified workflow described in this user guide includes:

- Enrichment as described in “Enrich a broad range of food samples” on page 15 and “Enrich production environment samples” on page 17
- SureTect™ Listeria monocytogenes PCR Assay

- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 12 for characteristics) with RapidFinder™ Analysis Software v2.0 or later and Pathogen Assay File: **ListeriaMono_SureTect_QS5** version 2.0 or later

Table 12 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 13 for characteristics) with RapidFinder™ Express Software v2.0 or later and Pathogen Assay File: **Listeria_monocytogenes_SureTect_7500_2.0** or later

Table 13 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 28

Table 14 Validated matrices

Matrices[1]
25 g bagged lettuce
25 g blue cheese
25 g cantaloupe melon
25 g cooked prawns
25 g cooked sliced ham
25 g cooked sliced turkey
25 g cottage cheese
25 g fresh bagged spinach
25 g ice cream
25 g pasteurized 2% milk
25 g pasteurized brie cheese
25 g pork Frankfurters
25 g processed cheese
25 g raw cod
25 g raw ground / minced beef
25 g raw ground / minced pork
25 g raw ground / minced turkey
25 g raw pork sausage
25 g salami
25 g smoked salmon
25 g deli salad (Piemontaise)
25 g pork rillettes
25 mL raw milk
25 g ready-to-cook vegetables
25 g greek yogurt
Ceramic environmental surface
Plastic environmental surface
Stainless steel surface
Concrete surface
25 mL process water

[1] Validated using the EN ISO 11290 method.

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 DNA*Zap*™ 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**.

Symbol definitions

Symbol	Definition
LOT	BATCH CODE
REF	CATALOG NUMBER
Σ	CONTAINS SUFFICIENT FOR <n> TESTS
	CONSULT INSTRUCTIONS FOR USE

(continued)

Symbol	Definition
	MANUFACTURER
	UPPER AND LOWER TEMPERATURE LIMIT (storage temperature)
	USE BY

C

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**.

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.

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; found at:
  www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/publications/i/item/9789240011311

D 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**

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, then select the appropriate country from the dropdown list.

Customer and technical support

Visit **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

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

Document	Publication number
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4378657
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide	4387777
SimpliAmp™ Thermal Cycler User Guide	MAN0009889
SimpliAmp™ Thermal Cycler Installation and Operation Quick Reference	A24827
RapidFinder™ Express Software Quick Reference	4480999

References

EN ISO 11290-1:2017. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. Part 1: Detection method.

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.

ISO/FDIS 18593:2018. Microbiology of the food chain – Horizontal methods for surface sampling.

EN ISO 16140-6:2016. Microbiology of food and animal feed – Method validation – Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.

FDA Bacteriological Analytical Manual (BAM), Chapter 10 - Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods

USDA/FSIS Microbiology Laboratory Guidebook, Method 8.11 - Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Ready-To-Eat Siluriformes (Fish) and Egg Products, and Environmental Samples

Guidelines Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, version 2012.