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# SureTect™ Salmonella species PCR Assay

## USER GUIDE

Lysis and real-time PCR detection of *Salmonella* species in food and environmental samples

for use with:

Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument with Thermo Scientific™ RapidFinder™ Analysis Software v1.2 or later

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

**Catalog Number** PT0100A

**Publication Number** MAN0017724

**Revision** E.0

PERFORMANCE TESTED
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For testing of Food and Environmental samples only.

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**Revision history:** Pub. No. MAN0017724

| Revision | Date | Description |
|---|---|---|
| E.0 | 17 February 2022 | A correction was made to the PCR materials: AB0771W replaced AB1800. |
| D.0 | 16 November 2021 | • AOAC First Action *Official Methods*℠ (No. 2021.02) certification was added.<br>• Spinning guidelines were added to the Procedural Guidelines.<br>• The RapidFinder™ Analysis Software version was updated throughout.<br>• Broad range designation and EN ISO 16140-6:2016 were added.<br>• Chocolate and cocoa matrices were added to Enrich food samples - standard protocol.<br>• Instructions for eliminating cross contamination were added to Test control organisms. |
| C.0 | 08 December 2020 | Updated software versions. Clarify workflow to indicate either type of PCR instrument can be used regardless of how lysate is prepped. |
| B.0 | 15 Oct 2019 | Removed references to Boekel Scientific heating blocks.<br>Updated Pathogen Assay File names. |
| A.0 | 12 December 2018 | Initial release with new publication number. Supersedes Pub. No. D11908 Version 10.<br>Additional changes:<br>• Includes the complete AOAC Research Institute *Performance Tested Methods*℠ -certified workflow that covers enrichment, DNA isolation, and real-time PCR detection.<br>• Added instructions for PCR on the QuantStudio™ 5 Instrument.<br>• Added Windows™ 10 software support.<br>• Corrected assay kit file references.<br>• Added a mixing step to ensure pellet is re-hydrated, in "Set up the PCR reactions."<br>• Removed information about the PikoReal™ Real-Time PCR System.<br>• Updated to the current document template, with associated changes in document organization, licensing, trademarks, and logos. |

# 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™ Salmonella species PCR Assay enables real-time PCR detection and differentiation of *Salmonella* species from food and production environment samples. This kit is for use in laboratories undertaking microbiological analysis and is 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 v1.2 or later | **SalmonellaSpp_SureTect_QS5** version 2.1 or later[1] |
| Applied Biosystems™ 7500 Fast Real-Time PCR Instrument | Applied Biosystems™ RapidFinder™ Express Software v2.0 or later | **Salmonella species SureTect** 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 *Salmonella* species 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 and Proteinase K to lyse bacterial cells present in the sample and release their DNA into solution.

Lysates are transferred to the SureTect™ Salmonella species 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 28 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 1 SureTect™ Salmonella species PCR Assay, 96 tests (Cat. No. PT0100A)**

| 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™ Salmonella species PCR Tubes | 12 strips of 8 tubes<br>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.

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

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

## Materials for enrichment

**Table 2 Equipment, accessories, and consumables**

| Item | Source |
|---|---|
| Homogenizer Laboratory Blender or Dilutor, one of the following or equivalent | DB5000A<br>DB4100A<br>DB4150A |
| Homogenizer bags appropriate for the sample type and size, one of the following or equivalent | For DB4100A or DB4150A:<br>DB4011A<br>DB4012A<br>DB4013A<br>DB4014A or equivalent |
| Incubators fitted with racks for homogenizer bags, set to 34°C to 38°C, and 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 or equivalent |
| Oxoid™ Novobiocin Selective Supplement[1] | SR0181E or equivalent |
| Oxoid™ Modified Tryptone Soya Broth[2] | CM0989B or equivalent |
| Cocoa Sample Recovery (CSR) Broth[3] | CM1155B |

[1] Validated for testing sprouted seeds.
[2] Validated for testing 375 g raw ground beef and beef trim samples.
[3] Validated for testing chocolate matrices.

**Table 4 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<br>• Swabs: MLS |

## Materials for lysis

**Table 5 Materials for lysis of enriched cultures**

| Item | Source |
|---|---|
| **Plastics, consumables, and reagents** | |
| Single-channel pipette, 10- to 100-µL<br>*or*<br>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 6 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 v1.2 or later<br>For use with SureTect™ Salmonella species PCR Assay and Pathogen Assay File: **SalmonellaSpp_SureTect_QS5** version 2.1 or later | A36320 (desktop)<br>A36328 (laptop)<br>Contact your local microbiology sales representative |

**Table 6 Materials for PCR** *(continued)*

| Item | Source |
|---|---|
| 7500 Fast Real-Time PCR Instrument with RapidFinder™ Express Software v2.0 or later<br>For use with SureTect™ Salmonella species PCR Assay and Pathogen Assay File: **Salmonella species SureTect** 2.0 or later | A30304 (desktop)<br>A30299 (laptop)<br>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, and one of the following plate holders with accessories: | PT0695 |
| **Precision Plate holder for SureTect™ assays** | PT0690 |
| Low Profile Tubes, strips of 8, white[1] | AB0771W |
| Ultra Clear qPCR Caps, strips of 8[1] | AB0866 |
| **7500 Fast Precision Plate Holder, for 0.1 mL tube strips** | A29252 |
| MicroAmp™ Fast 8-Tube Strip, 0.1 mL[2] | 4358293 |
| MicroAmp™ Optical 8-Cap Strips[2] | 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 7 Materials for confirmation of positive results**

| Item | Source |
|---|---|
| Oxoid™ *Brilliance*™ Salmonella Agar | • CM1092B (base)<br>• SR0194E (supplement)[1]<br>Outside North America: R110374 (monoplate) |
| Oxoid™ Rappaport-Vassiliadis Soya (RVS) Peptone Broth | CM0866B |
| XLD Agar | CM0469B or equivalent[1] |
| Saline | MLS |
| **For a miniaturized biochemical identification panel** | |
| Oxoid™ Salmonella Test Kit (latex test) | DR1108A |
| Microbact™ GNB 24E Kit, 40 tests | MB1131A |
| Microbact™ GNB Reagent Set D | MB1082A |

[1] Contact your Thermo Scientific Microbiology sales representative for prepared media options.

# Validated workflows

Enrich food samples—short protocol (page 14)

*or*

Enrich food samples—broad range of foods (page 15)

*or*

Enrich production environment samples (page 17)

▼

Prepare the lysate using the thermal cycler method (page 18)

▼

*or*

Prepare the lysate using the heat block method (page 20)

▼

PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v1.2 or later (page 21)

*or*

PCR with the 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later (page 23)

▼

Confirm positive results (page 26)

# 2 Before you begin

## Procedural guidelines

### Guidelines for sample enrichment

- For preparation of master suspensions, follow the instructions of EN ISO 6887:2017 series and EN ISO 6579:2017 standards. Comply with Good Laboratory Practices (refer to EN ISO 7218:2007 standard).
- Follow the manufacturer's instructions for preparation of culture media.
- When following the short enrichment protocol, ensure that the enrichment broth is pre-warmed for 18–24 hours before adding to the sample.
- 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.
- According to EN ISO 6887-4:2017, for the chocolate and cocoa category, Non-Fat Dried Milk could be replaced with UHT Milk, ensure the addition to the enrichment broth of the following:
  - Brilliant green (0.018 g/L) for products with high background flora.
  - Tween™ 80 for the products with >20% fat content.

### 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

- PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
- **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.
- Perform all centrifugation steps as described in the procedure to ensure optimal reaction conditions.
- 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 34. For more information go to **www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html**.

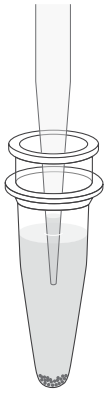


**Figure 1 Avoid lysis particles**

## 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 recommended before 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 food samples—short protocol

1. Pre-warm the indicated volume of media to 41.5±1°C.
2. Transfer the food sample to a homogenizer bag, then add the pre-warmed media, as indicated.

**Table 8 Enrichment conditions—short protocol**

| Matrices | PCR instrument | Media | Incubation |
|---|---|---|---|
| 25 g raw ground beef | • QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v1.2 or later<br>• 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later | 1-in-10 ratio of sample to media<br>For example, 25 g of sample and 225 mL of pre-warmed Buffered Peptone Water (ISO) | 41.5±1°C for 8–24 hours |
| 375 g raw ground beef | | 1-in-5 ratio of sample to media<br>For example, 375 g of sample and 1.5 L of pre-warmed Oxoid™ Modified Tryptone Soya Broth | 41.5±1°C for 9–24 hours |
| 375 g beef trim | | 1-in-5 ratio of sample to media<br>For example, 375 g of sample and 1.5 L of pre-warmed Oxoid™ Modified Tryptone Soya Broth | 41.5±1°C for 8–24 hours |
| 375 g spinach | | 1-in-5 ratio of sample to media<br>For example, 375 g of sample and 1.5 L of pre-warmed Buffered Peptone Water (ISO) | 41.5±1°C for 8–24 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 8.

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.

# Enrich food samples—broad range of foods

1. Transfer the food sample to a homogenizer bag, then add room-temperature media, as indicated.

**Table 9 Enrichment conditions**

| Matrices | PCR instrument | Media | Incubation |
|---|---|---|---|
| **Standard protocol for a broad range of foods** | | | |
| 25 g Non-fat dried milk powder (NFDM), raw ground beef, and liquid whole egg | • QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v1.2 or later<br>• 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later | 1-in-10 ratio of sample to media<br>For example, 25 g of sample and 225 mL of Buffered Peptone Water (ISO) | 34°C to 38°C for 18–26 hours |
| 25 g Cooked shrimp, pork Frankfurters, lettuce, chilled ready to eat meal (containing beef), raw ground pork, raw chicken breast, wet cat food, dry dog food, pasteurized milk, cantaloupe melon, chilled pizza dough, black peppercorns, ice cream and peanut butter, cut cabbage, cut mango, grated cheese, grated cheddar cheese, feta cheese, cream | | 1-in-10 ratio of sample to media<br>For example, 25 g of sample and 225 mL of Buffered Peptone Water (ISO) | 34°C to 38°C for 20–28 hours |
| Protocols for specific matrices and large test portions | | | |

**Table 9 Enrichment conditions** *(continued)*

| Matrices | PCR instrument | Media | Incubation |
|---|---|---|---|
| 25 g Sprouted seeds | • QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v1.2 or later<br>• 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later | 1-in-10 ratio of sample to media<br>For example, 25 g of sample and 225 mL of pre-warmed Buffered Peptone Water (ISO) with 20 mg/L novobiocin | 41.5±1°C for 20–28 hours |
| 25 g Chocolate | | 1-in-10 ratio of sample to media For example, 25 g of sample and 225 mL of Cocoa Sample Recovery (CSR) Broth | 34°C to 38°C for 20–28 hours |
| Up to 375 g Cocoa powder, Cocoa liquor, Cocoa butter, Dark chocolate (>70%) | | 1-in-10 ratio of sample to media For example, 375 g of sample and 3,375 mL of pre-warmed Buffered Peptone Water (ISO) or pre-warmed NFDM | 34°C to 38°C for 22–30h (for BPW) or<br>34°C to 38°C for 20–28h (for NFDM) |

2. 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.
3. Incubate as described in Table 9.
4. 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 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. Add samples to media, as indicated.

| Sample type | Volume of media/sample | Media |
|---|---|---|
| Swabs | 10 mL | Buffered Peptone Water (ISO) at room temperature |
| Sponges | 100 mL | |

2. Homogenize thoroughly.

3. Incubate at 34°C to 38°C for 18–26 hours.

4. 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.

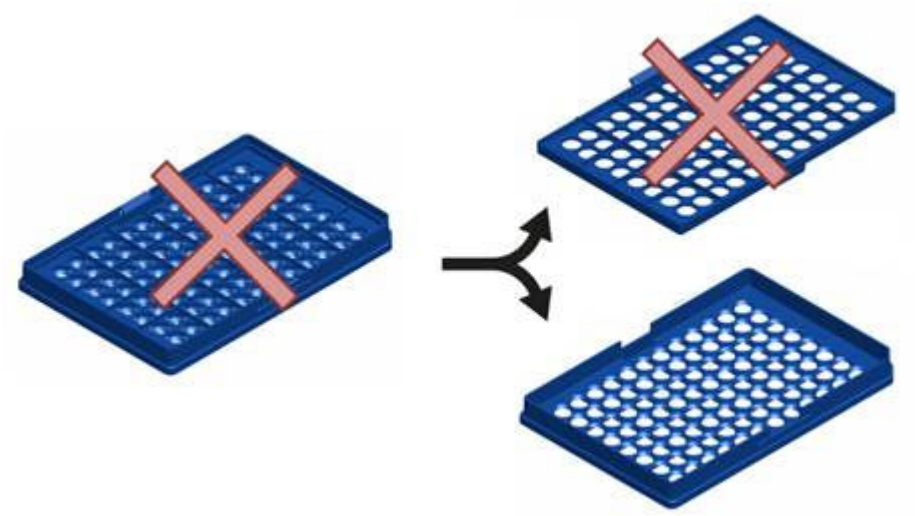
   These tubes are referred to as Lysis Tubes in the rest of the procedure.

   **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. For chocolate and cocoa samples only, dilute the enrichment 1-in-5 (1 part enriched sample and 4 parts sterile BPW).
4. Transfer 10 µL of the enriched sample (or diluted chocolate/cocoa samples) 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 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").



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.

   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. For chocolate and cocoa samples only, dilute the enrichment 1-in-5 (1 part enriched sample and 4 parts sterile BPW).
5. Transfer 10 µL of the enriched sample (or diluted chocolate/cocoa samples) 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.
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 v1.2 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 the RapidFinder™ Analysis Software, click **Create Experiment**, then enter or edit the well parameters.

Select **SalmonellaSpp_SureTect_QS5** version 2.1 or later for the assay.

### Set up the PCR reactions

Before starting this procedure, see the "Guidelines for PCR" on page 13.

1. Following the plate layout previously set up in the software, place the required number of SureTect™ Salmonella species 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 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 more than one strip of PCR tubes are required, then repeat steps 2–5.

7. Mix all PCR tubes thoroughly by vortexing for 10–15 seconds to ensure that the pellet is fully rehydrated.

8. Spin the PCR tubes for at least 10 seconds to remove bubbles and to collect the liquid at the bottom of the tube before placing in the PCR instrument.

**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 **Salmonella species SureTect** 2.0 or later for the assay.

## Set up the PCR reactions

Before starting this procedure, see the "Guidelines for PCR" on page 13.

1. Following the plate layout previously set up in the software, place the required number of SureTect™ Salmonella species 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 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 more than one strip of PCR tubes are required, then repeat steps 2–5.

7. Mix all PCR tubes thoroughly by vortexing for 10–15 seconds to ensure that the pellet is fully rehydrated.

8. Spin the PCR tubes for at least 10 seconds to remove bubbles and to collect the liquid at the bottom of the tube before placing in the PCR instrument.

**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.

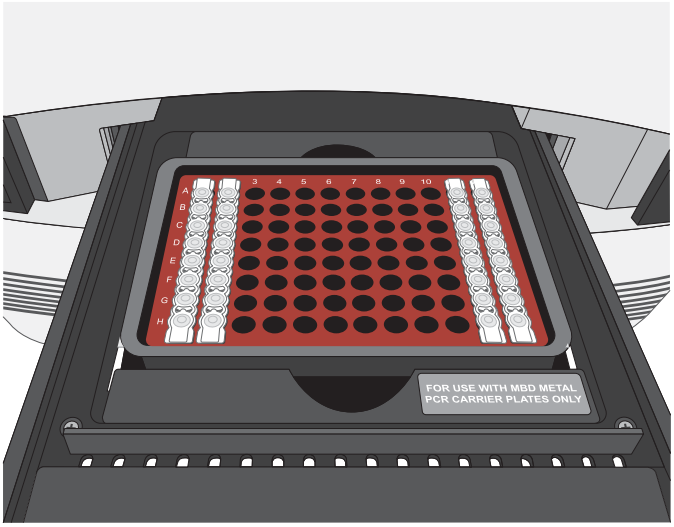



**Figure 2 7500 Fast instrument tube layout**

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 to report, export, and store results.

# 6 Confirm positive results

## Recommended confirmation methods

In the context of AOAC *Performance Tested Methods*℠ certification and the AOAC First Action *Official 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 or biochemical galleries (see "Isolate presumptive positives" on page 26).
- The appropriate reference method for the sample matrix (see "AOAC *Performance Tested Methods*℠ and AOAC First Action *Official Methods*℠ Certification" on page 31).

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*™ Salmonella Agar, then incubate for 22–26 hours at 34°C to 38°C.

   **Note:** XLD can replace *Brilliance*™ Salmonella Agar for cocoa and chocolate products.

2. (*Optional*) If there is high background on the plate, perform additional selective plating:
   a. Transfer 0.1 mL of the stored enriched culture to 10 mL of Oxoid™ Rappaport-Vassiliadis Soya (RVS) Peptone Broth (Cat. No. CM0866B), then incubate at 41.5±1°C for 21–27 hours.

   b. Streak 10 µL of the incubated RVS Broth onto *Brilliance*™ Salmonella Agar, then incubate at 34°C to 38°C for 22–26 hours.

      **Note:** XLD can replace *Brilliance*™ Salmonella Agar for cocoa and chocolate products.

3. Confirm well-isolated, presumptive positive *Salmonella* colonies using the Oxoid™ Salmonella Test Kit (latex test), Microbact™ GNB 24E Kit, or depending on the legislation territory, an appropriate Official Method of Analysis of AOAC International (AOAC-OMA) or EN ISO 16140-6:2019 validated confirmation method, or any appropriate reference confirmation procedure (e.g. FDA/BAM Chapter 5, USDA/FSIS MLG 4.10, EN ISO 6579:2017 standard).

   Some strains of *Salmonella* ser. Dublin may show weak pigmentation on *Brilliance*™ Salmonella Agar, because of their low esterase activity.

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 and clean pipetting equipment.<br>2. If the negative extraction control continues to show contamination, repeat the assay using a new kit.<br>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:<br>• Preferential amplification of the carryover DNA.<br>• Carryover of particles from the Lysis Tube. | 1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.<br>2. If the negative extraction control continues to show contamination, repeat the assay using a new kit.<br>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 and/or lysate is not at the bottom of the tube before starting the PCR run. | 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 cause 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:<br>• Carryover of particles from the Lysis Tube.<br>• PCR inhibitors present in the food sample.<br>• Incomplete sample lysis.<br>• 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 are not fully dissolved and/or lysate is not at the bottom of the tube before starting the PCR run. | 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 are 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 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 *Brilliance*™ Salmonella Agar are not present | Overgrowth of *Salmonella* with background flora. | Sub-culture 100 µL of the retained enrichment into 10 mL of Rappaport-Vassiliadis Soya (RVS) Broth. Incubate at 41.5 °C for 21–27 hours before continuing with confirmation. |
| In confirmation testing, suspect colonies on *Brilliance*™ Salmonella Agar are too small to conduct a latex test and serological confirmation | 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. |
| In confirmation testing, suspect colonies on *Brilliance*™ Salmonella Agar are not well isolated | The enriched sample contains high levels of background flora that were not inhibited on *Brilliance*™ Salmonella Agar. | Purify the suspect colonies on a second *Brilliance*™ Salmonella Agar plate before continuing with confirmation. |

# 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 some target isolate 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 recommended by Thermo Fisher Scientific, Microbiology Division.
  - 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 instrument (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 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™ Salmonella species PCR Assay | 50 | 45 |

# B Supplemental information

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

Go to **thermofisher.com/foodsafety** for a guide to workflows for detection of *Salmonella* spp. (Pub. No. MAN0009417).

**Table 10 *Performance Tested Methods*℠ and *Official Methods*℠ Certification of the workflow**

| *Performance Tested Methods*℠ Certification | *Official Methods*℠ Certification |
|---|---|
| PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 051303 | AOAC First Action *Official Methods*℠ (No. 2021.02)<br>**https://www.aoac.org/scientific-solutions/standards-and-official-methods/** |

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

- Enrichment as described in "Enrich food samples—short protocol" on page 14 and "Enrich production environment samples" on page 17
- SureTect™ Salmonella species PCR Assay
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 11 for characteristics) with RapidFinder™ Analysis Software v1.2 or later and Pathogen Assay File: **SalmonellaSpp_SureTect_QS5** version 2.1 or later

**Table 11 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<br>Maximum: 9.0°C/sec |
| Thermal range | 4–99°C |
| Thermal accuracy | ±0.25°C |

**Table 11 QuantStudio 5 Real-Time PCR Instrument characteristics** *(continued)*

| Characteristics | QuantStudio™ 5 Real-Time PCR Instrument |
|---|---|
| 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 12 for characteristics) with RapidFinder™ Express Software v2.0 or later and Pathogen Assay File: **Salmonella species SureTect** 2.0 or later

**Table 12 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<br>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 26

**Table 13 Validated matrices**

| Matrices |
|---|
| 25 g black peppercorns[1] |
| 25 g cantaloupe melon[1] |
| 25 g chilled pizza dough[1] |
| 25 g chilled ready to eat meal (containing beef)[1] |
| 25 g chocolate[1] |
| 25 g cooked shrimp[1] |
| 25 g cut cabbage[1,2] |
| 25 g cut mango[1,2] |
| 25 g dry dog food[1] |
| 25 g grated cheese[1,2] |
| 25 g grated cheddar cheese[1,2] |
| 25 g feta cheese[1,2] |

**Table 13 Validated matrices** *(continued)*

| Matrices |
|---|
| 25 g ice cream[1] |
| 25 g cream[1] |
| 25 g bagged lettuce[1] |
| 25 g liquid whole egg[1] |
| 25 g non-fat dried milk powder[1] |
| 25 g pasteurized 2% fat milk[1] |
| 25 g peanut butter[1] |
| 25 g pork Frankfurters[1] |
| 25 g raw chicken breast[1] |
| 25 g raw ground beef (standard and short enrichment protocol)[1] |
| 25 g raw ground pork[1] |
| 25 g sprouted seeds[1] |
| 25 g wet cat food[1] |
| 375 g beef trim[3] |
| 375 g raw ground beef (short enrichment protocol)[1] |
| 375 g spinach[2] |
| Up to 375 g dark chocolate (>70%)[1,2] |
| Up to 375 g cocoa liquor[1,2] |
| Up to 375 g cocoa butter[1,2] |
| Up to 375 g cocoa powder[1,2] |
| Plastic environmental surface[1] |
| Stainless steel surface[1] |

[1] Validated using the EN ISO 6579 method.
[2] Validated using the FDA/BAM Chapter 5 method
[3] Validated using the USDA/FSIS MLG 4.10 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). After cleaning with bleach we recommend a rinse with an ethanol solution because bleach will rust stainless steel.

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:
  **https://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: **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 |
|---|---|
| *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 |
| *PCR Starter Kit for 96-well blocks, 0.2 mL, User Guide* | A24829 |

# References


EN ISO 6579-1:2017. Microbiology of the food chain – Horizontal method for detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp.

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.

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