Real-time PCR Detection of *Salmonella* spp. in Food and Environmental Samples

**USER GUIDE**

*Performance Tested Methods*™-certified workflow

for use with:

- PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing
- KingFisher™ Flex-96 Deep Well Magnetic Particle Processor
- MagMAX™ Express-96 Deep Well Magnetic Particle Processor
- MicroSEQ™ Salmonella spp. Detection Kit
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software

Publication Number 4405968
Revision E
The information in this guide is subject to change without notice.

**DISCLAIMER:** TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

**Revision history:** Pub. No. 4405968 [English]

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
</table>
| E        | 01 March 2019  | • Changed storage temperature for Magnetic Particles from 4°C to room temperature, to reflect new specifications.  
• Reorganized procedural guidelines and made minor wording changes, for clarification or to align with current template organization, style, and format.  
• Add KingFisher™ Flex-96 instrument information.  
• Updated to a Thermo Fisher Scientific template, with associated updates to legal statements. |
| D        | November 2013  | • Added instructions for PCR with the MicroSEQ™ Salmonella spp. Detection Kit and confirmation testing.  
• Changed document title to reflect additional instructions.  
• Added logos and certification details for AOAC Performance Tested Methods™ certification.  
• Added additional troubleshooting tips.  
• Clarified instructions for handling Magnetic Particles, enriching samples in a chocolate matrix, and storage of DNA samples in the Elution Plate.  
• Updated number format (time, temperature, and centrifugation speeds) for AOAC certification.  
• Updated to a Life Technologies template, with associated updates to the limited license information, warranty information, and trademark statement. |
| C        | June 2010      | Corrected typographical error in the “Exclusions, Conditions, Exceptions, and Limitations” section. |
| B        | September 2009 | • Added instructions for handling Magnetic Particles, high-fat samples, and carryover of Magnetic Particles of food particulate residues in final eluates.  
• Updated limited license information, warranty information, and trademark statements. |

**Important Licensing Information:** These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

**Trademarks:** All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. AOAC is a trademark and Performance Tested Methods is a service mark of AOAC INTERNATIONAL. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license.

©2019 Thermo Fisher Scientific Inc. All rights reserved.
CHAPTER 1  Overview ................................................................. 5

CHAPTER 2  Enrich food or environmental samples ....................... 6
  Materials for enrichment of food samples ........................................... 6
  Enrich samples ............................................................................ 7

CHAPTER 3  Isolate DNA with the PrepSEQ™ Nucleic Acid
Extraction Kit ............................................................................. 9
  Product description ....................................................................... 9
  Materials for DNA Isolation .......................................................... 9
  Procedure overview ..................................................................... 11
    Workflow ............................................................................... 12
  Before first use of the kit ............................................................ 12
    Prepare Binding Solution and Wash Buffer .................................. 12
  Before each use of the kit ............................................................ 13
    Resuspend Magnetic Particles ................................................... 13
  Centrifuge the enriched culture .................................................... 13
    For large sample pellets: perform the preclarification protocol ......... 14
    For high-fat samples: remove fat layer before lysis ....................... 14
  Set up the Lysis Plate ................................................................. 14
  Set up the processing plates ....................................................... 15
  Process samples on the instrument ............................................... 15

CHAPTER 4  PCR with the MicroSEQ™ Salmonella spp.
Detection Kit and RapidFinder™ Express Software ....................... 17
  Product description ..................................................................... 17
  Materials for PCR detection ....................................................... 18
  Workflow ................................................................................... 19
  Important procedural guidelines for PCR ...................................... 20
    Software ............................................................................... 20
    Sample handling ..................................................................... 20
    MicroAmp™ tube strips ......................................................... 21
  Create or edit a run file in RapidFinder™ Express Software ............. 21
Prepare the assay beads ........................................................ 21
Set up the PCR reactions ........................................................ 22
Load and run the reactions ...................................................... 22
View results and data analysis ................................................... 23
  If necessary, investigate results in SDS Software ...................... 23

■ CHAPTER 5  Recommended confirmation methods ................. 24

■ APPENDIX A  Troubleshooting ......................................... 25

■ APPENDIX B  Supplemental information .......................... 29
  Sensitivity ........................................................................ 29
  Specificity ....................................................................... 29
  Operational conditions ...................................................... 29
  AOAC Performance Tested Methods™ Certification ................. 30
  Good laboratory practices for PCR ...................................... 30

■ APPENDIX C  Safety ...................................................... 32
  Chemical safety .................................................................. 33
  Biological hazard safety .................................................... 34

Documention and support ........................................................ 35
  Food Safety support .......................................................... 35
  Customer and technical support ........................................... 35
  Related documentation .................................................... 36

References ....................................................................... 37
This guide describes the following AOAC Performance Tested MethodsSM workflow for detection of Salmonella spp. in food and environmental samples:

1. Enrichment of 25-g or 25-mL samples in Buffered Peptone Water (BPW). Chocolate-based samples require BPW with skim milk powder and Brilliant Green dye.

2. Automated preparation of PCR-ready DNA using the PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing and the KingFisher™ Flex-96 Deep Well Magnetic Particle Processor or the MagMAX™ Express-96 Magnetic Particle Processor. The KingFisher™ Flex-96 Deep Well Magnetic Particle Processor or the MagMAX™ Express-96 Magnetic Particle Processor enable high-throughput sample processing in a 96-well format with minimal handling.


4. Confirmation testing of positive samples.

See “AOAC Performance Tested MethodsSM Certification” on page 30 for detailed information about the AOAC certification. See “Specificity” on page 29 for details about assay specificity.

This workflow is intended for use by microbiological analysts who need to test for Salmonella spp. in food or environmental samples. These kits are for use in food and environmental testing only. Not for any animal or human therapeutic or diagnostic use.

Go to thermofisher.com/foodsafety for a list of workflows for detection of Salmonella spp.
Enrich food or environmental samples

Materials for enrichment of food samples

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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equipment</strong></td>
<td></td>
</tr>
<tr>
<td>Incubator, 30±1°C</td>
<td>MLS</td>
</tr>
<tr>
<td><strong>Homogenizer Laboratory Blender, one of the following or equivalent</strong></td>
<td></td>
</tr>
<tr>
<td>Homogenizer Laboratory Blender</td>
<td>DB5000A, CM1211B, or equivalent</td>
</tr>
<tr>
<td>Diluflux™ Pro Automated Gravimetric Dilutor</td>
<td>DB4150A, DB4100A, or equivalent</td>
</tr>
<tr>
<td><strong>Consumables</strong></td>
<td></td>
</tr>
<tr>
<td>For environmental samples only:</td>
<td></td>
</tr>
<tr>
<td>Swab, cotton</td>
<td>MLS</td>
</tr>
<tr>
<td>15-mL conical tubes</td>
<td>MLS</td>
</tr>
<tr>
<td><strong>Homogenizer bag appropriate for the sample type</strong></td>
<td></td>
</tr>
<tr>
<td>Homogenizer bag BagFilter™ 400</td>
<td>DB4011A, or equivalent</td>
</tr>
<tr>
<td>Homogenizer bag BagPage™ 400</td>
<td>DB4012A, or equivalent</td>
</tr>
<tr>
<td>Homogenizer bag BagLight™ 400</td>
<td>DB4013A, or equivalent</td>
</tr>
<tr>
<td>Homogenizer bag BagLight™ 1300</td>
<td>DB4014A, or equivalent</td>
</tr>
</tbody>
</table>
## Reagents

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffered Peptone Water (BPW)</td>
<td>DF1049, or equivalent</td>
</tr>
<tr>
<td>Skim milk powder (for chocolate samples only)</td>
<td>LP0031B, or equivalent</td>
</tr>
<tr>
<td>Brilliant Green Dye Solution (for chocolate samples only)</td>
<td>MLS</td>
</tr>
<tr>
<td>Dey Engley (D/E) Neutralizing Broth (for environmental swabs or sponges)</td>
<td>BD 281910, CM1029B, R453052, or equivalent</td>
</tr>
</tbody>
</table>

## Enrich samples

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

1. Prepare Buffered Peptone Water (BPW) according to the manufacturer’s instructions.

   **Note:** For growth of bacteria in a chocolate matrix, prepare BPW containing 100 g/L of sterile skim milk powder, which reduces the growth inhibition characteristic of chocolate.

2. Combine BPW and sample as described in the following table.

<table>
<thead>
<tr>
<th>Sample type [1]</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>Add 225 mL of BPW (+ skim milk powder for chocolate samples) to 25 g (or 25 mL) of sample.</td>
</tr>
</tbody>
</table>
| Environmental swab | 1. Prewet the swab with 0.5 mL of D/E Neutralizing Broth.  
                     2. Wipe the surface area to be tested.  
                     3. Add the swab to 10 mL of BPW in a 15-mL conical tube. |
| Environmental sponge | 1. Prewet the sponge with 10 mL of D/E Neutralizing Broth.  
                          2. Wipe the surface area to be tested.  
                          3. Add the sponge to 100 mL of BPW. |

[1] See Table in “AOAC Performance Tested Methods” Certification” on page 30
3. Mix the sample with BPW as described in the following table:
   A filtered bag may be used for enrichment of samples with particulates.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food:</td>
<td></td>
</tr>
<tr>
<td>• Coarse food types,</td>
<td>Homogenize the sample thoroughly using a blender.</td>
</tr>
<tr>
<td>such as meat, poultry,</td>
<td></td>
</tr>
<tr>
<td>and seafood[1]</td>
<td></td>
</tr>
<tr>
<td>• Eggs</td>
<td></td>
</tr>
<tr>
<td>Food:</td>
<td></td>
</tr>
<tr>
<td>Liquids or powdered</td>
<td>Shake the bag at least 25 times to achieve a homogeneous suspension.</td>
</tr>
<tr>
<td>Environmental swab</td>
<td>Twirl the swab for 90±30 seconds.</td>
</tr>
<tr>
<td>Environmental sponge</td>
<td>Use one of the following methods:</td>
</tr>
<tr>
<td></td>
<td>• Use a nonfiltered, nonmesh enrichment bag and homogenize the sample thoroughly using a blender.</td>
</tr>
<tr>
<td></td>
<td>• Hand squeeze for about 1 minute.</td>
</tr>
</tbody>
</table>

[1] Hand massage foods that cannot be processed in a homogenizer.

4. Incubate at 37±1°C under static conditions for 16–20 hours.

**Note:** For growth of bacteria in a chocolate matrix:
1. Incubate at 37±1°C for 1–2 hours.
2. Add Brilliant Green Dye Solution to a final concentration of 0.018 g/L.
3. Continue to incubate at 37±1°C for a total of 16–20 hours.
Product description

The PrepSEQ™ Nucleic Acid Extraction Kit (Cat. nos. 4428176, 4480466) is designed for preparation of high-quality microbial nucleic acid from broth cultures. Magnetic beads allow efficient DNA capture and sample washing. The kit enables high-throughput automation in a 96-well plate format on the KingFisher™ Flex-96 Deep Well Magnetic Particle Processor, or the MagMAX™ Express-96 Magnetic Particle Processor. Go to thermofisher.com/foodsafety for a list of workflows with non-automated DNA isolation methods.

Materials for DNA Isolation

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.

Table 2  PrepSEQ™ Nucleic Acid Extraction Kit

<table>
<thead>
<tr>
<th>Contents</th>
<th>Cat. No. 4480466 (100 reactions)</th>
<th>Cat. No. 4428176 (300 reactions)</th>
<th>Storage[1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Buffer</td>
<td>2 × 50 mL</td>
<td>6 × 50 mL</td>
<td></td>
</tr>
<tr>
<td>Magnetic Particles</td>
<td>2 × 1.5 mL</td>
<td>6 × 1.5 mL</td>
<td></td>
</tr>
<tr>
<td>Binding Solution (Isopropanol)[2]</td>
<td>1 empty bottle</td>
<td>3 empty bottles</td>
<td>15°C to 30°C</td>
</tr>
<tr>
<td>Wash Buffer Concentrate[3]</td>
<td>2 × 26 mL</td>
<td>6 × 26 mL</td>
<td></td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>25 mL</td>
<td>3 × 25 mL</td>
<td></td>
</tr>
</tbody>
</table>
### Chapter 3 Isolate DNA with the PrepSEQ™ Nucleic Acid Extraction Kit

#### Materials for DNA Isolation

<table>
<thead>
<tr>
<th>Contents</th>
<th>Cat. No. 4480466 (100 reactions)</th>
<th>Cat. No. 4428176 (300 reactions)</th>
<th>Storage[1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinase K (PK) Buffer</td>
<td>50 mL</td>
<td>3 × 50 mL</td>
<td>15°C to 30°C</td>
</tr>
<tr>
<td>Proteinase K, 20 mg/mL</td>
<td>1.25 mL</td>
<td>3 × 1.25 mL</td>
<td>–25°C to –15°C</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KingFisher™ Flex-96 instrument and accessories</strong></td>
<td></td>
</tr>
<tr>
<td>KingFisher™ Flex-96 Deep Well Magnetic Particle Processor</td>
<td>A32681, 96 DW plate, or equivalent[1]</td>
</tr>
<tr>
<td>KingFisher™ Deepwell 96 Plate, V-bottom</td>
<td>95040450</td>
</tr>
<tr>
<td>KingFisher™ 96 KF microplates (200 µL)</td>
<td>97002540</td>
</tr>
<tr>
<td>KingFisher™ Flex 96 KF Heating Block</td>
<td>24075420</td>
</tr>
<tr>
<td>KingFisher™ 96 tip comb for DW magnets</td>
<td>97002534</td>
</tr>
<tr>
<td>Finntip™ Filtered Pipette Tips</td>
<td>94052320, or equivalent</td>
</tr>
<tr>
<td><strong>MagMAX™ Express-96 instrument and accessories</strong></td>
<td></td>
</tr>
<tr>
<td>MagMAX™ Express-96 Deep Well Magnetic Particle Processor</td>
<td>Contact your local sales representative.</td>
</tr>
<tr>
<td>MagMAX™ Express-96 Deep Well Plates</td>
<td>4388476</td>
</tr>
<tr>
<td>MagMAX™ Express-96 Standard Plates</td>
<td>4388475</td>
</tr>
<tr>
<td>MagMAX™ Express-96 Deep Well Tip Combs</td>
<td>4388487</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equipment</strong></td>
<td></td>
</tr>
<tr>
<td>Benchtop microcentrifuge</td>
<td>Eppendorf 5415 D or equivalent</td>
</tr>
<tr>
<td>96-Well Magnetic-Ring Stand</td>
<td>AM10050</td>
</tr>
<tr>
<td>Block heater, 37°C</td>
<td>MLS</td>
</tr>
<tr>
<td>Laboratory mixer, Vortex or equivalent</td>
<td>MLS</td>
</tr>
</tbody>
</table>
### Procedure overview

1 mL of enriched sample is centrifuged. If the enriched sample produces a large food pellet, a fresh aliquot of the enriched sample is processed using the preclarification protocol. The sample pellet is lysed and processed on the instrument. Then, Binding Solution and Magnetic Particles are added to the samples, and after washing by the instrument, nucleic acid is eluted with 140 µL of Elution Buffer. The eluted DNA sample is ready for downstream PCR.
## Workflow

### Centrifuge sample after enrichment

Centrifuge 1 mL of sample at 12,000–16,000 \( \times g \) for about 3 minutes.

- If no large pellet: remove and discard the supernatant.
- If a large pellet: perform the preclarification protocol on a fresh, 1-mL aliquot of the enriched sample.

### Set up the Lysis Plate

Resuspend pellet in 300 µL of Lysis Buffer.

Transfer the sample to a Deep Well Plate (Lysis Plate).

### Set up the processing plates

- Tip Comb plate: Standard plate + Deep Well Tip Comb
- Elution Plate: 140 µL Elution Buffer
- Wash Plate 1: 300 µL Wash Buffer
- Wash Plate 2: 300 µL Wash Buffer

### Process samples on the instrument

Select a program, select **Start**, and load processing plates into the instrument.

After 18 minutes, add 30 µL of Magnetic Particles and 180 µL of Binding Solution. Reload the plate, and press **Start**.

When the run is complete, DNA is in Elution Buffer (Elution Plate).

### Proceed to PCR, or seal the plate and store the DNA below –18°C

## Before first use of the kit

### Prepare Binding Solution and Wash Buffer

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

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

Resuspend Magnetic Particles

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

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

1. Incubate the tube of Magnetic Particles at 37±1°C for approximately 10 minutes.
2. Vortex for approximately 10 seconds.
   
   **Note:** If the white precipitate is not completely dissolved after 10 minutes at 37°C, apply longer incubation times and higher temperatures (up to 50°C).
3. Keep at room temperature (23±5°C) until ready for use.

Centrifuge the enriched culture

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

1. Transfer 1 mL of enriched culture to a 1.5-mL microcentrifuge tube.
2. Centrifuge the tube at 12,000–16,000 × g for about 3 minutes.
   
   (Optional) If the sample creates a large pellet, follow “For large sample pellets: perform the preclarification protocol” on page 14 with a fresh sample of the enriched culture.
3. Remove and discard the supernatant as quickly as possible to prevent dissipation of pellet.
   
   (Optional) If necessary, follow “For high-fat samples: remove fat layer before lysis” on page 14.
For samples that produce a large pellet upon initial centrifugation, follow this preclarification protocol:

1. Transfer a fresh, 1 mL sample of enriched culture to a 1.5-mL microcentrifuge tube.

2. Centrifuge the tube containing the sample at about $4000 \times g$ for about 1 minute.

3. Transfer the supernatant to a new 1.5-mL microcentrifuge tube without disturbing the pellet. Discard the pellet.

4. Centrifuge the tube containing the supernatant at 12,000–16,000 $\times g$ for about 3 minutes.

5. Remove and discard the supernatant as quickly as possible to prevent dissipation of pellet.  
   (Optional) If necessary, follow “For high-fat samples: remove fat layer before lysis” on page 14.

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

<table>
<thead>
<tr>
<th>Type of fat layer</th>
<th>Fat layer and supernatant removal</th>
</tr>
</thead>
</table>
| Liquid            | 1. Use a P1000 pipettor to remove fat from the top surface by aspirating in a circular motion without disturbing the pellet.  
   2. Continue to collect supernatant from the top surface until all the supernatant is removed.  
   3. Discard the supernatant into a waste container. |
| Solid             | 1. Use a pipette tip to gently dislodge the fat layer without disturbing the pellet.  
   2. Aspirate the supernatant from the top surface using a pipettor until all the supernatant is removed.  
   3. Discard the supernatant into a waste container. |

Set up the Lysis Plate

1. Add 300 µL of the Lysis Buffer to the tube.

2. Resuspend by pipetting up and down.  
   Alternatively, vortex until the pellet is resuspended, and quick spin for about 5 seconds to remove the Lysis Buffer from the tube lid.

3. Transfer the sample to a deep well Lysis Plate.

4. (Optional but recommended) Set up a negative extraction control (NEC) containing 300 µL of Lysis Buffer in the Lysis Plate.
Set up the processing plates

Set up the processing plates as described in the following table.

<table>
<thead>
<tr>
<th>Plate</th>
<th>Plate type</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip Comb</td>
<td>Standard</td>
<td>Place a 96-well Deep Well Tip Comb in a standard plate.</td>
</tr>
<tr>
<td>Elution Plate</td>
<td>Standard</td>
<td>Add 140 µL of Elution Buffer to each sample and control well.</td>
</tr>
<tr>
<td>Wash Plate 1</td>
<td>Deep Well</td>
<td>Add 300 µL of Wash Buffer to each sample and control well.</td>
</tr>
<tr>
<td>Wash Plate 2</td>
<td>Deep Well</td>
<td>Add 300 µL of Wash Buffer to each sample and control well.</td>
</tr>
</tbody>
</table>

Process samples on the instrument

1. Power on the instrument, select the program, and press Start.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Program</th>
</tr>
</thead>
<tbody>
<tr>
<td>KingFisher™ Flex-96</td>
<td>4412639PrepSEQ_Sal</td>
</tr>
<tr>
<td>MagMAX™ Express-96</td>
<td>44000799DWPrepSEQGN</td>
</tr>
</tbody>
</table>

2. Load the prepared plates according to the readout on the instrument, verifying that their orientation is [A1 to A1].

<table>
<thead>
<tr>
<th>Plate</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip Comb</td>
<td>Load the tip comb, then press Start.</td>
</tr>
<tr>
<td>Elution Plate</td>
<td>Load the Elution Plate, then press Start.</td>
</tr>
<tr>
<td>Wash Plate 2</td>
<td>Load Wash Plate 2, then press Start.</td>
</tr>
<tr>
<td>Wash Plate 1</td>
<td>Load Wash Plate 1, then press Start.</td>
</tr>
<tr>
<td>Lysis Plate</td>
<td>Load Lysis Plate, then press Start.</td>
</tr>
</tbody>
</table>

3. Dispense the Magnetic Particles and Binding Solution, when prompted by the instrument (after ~18 minutes).
   a. Vortex the Magnetic Particles for about 10 seconds until resuspension is complete.
   b. Add 30 µL of Magnetic Particles to each well.
   c. Add 180 µL of Binding Solution to each well.
   d. Load the plate back into the instrument. Press Start.

4. When DNA sample preparation is complete (“Enjoy your DNA” is displayed on the screen), remove the Elution Plate from the instrument. The Elution Plate contains DNA in Elution Buffer.
5. Proceed directly to real-time PCR, or store the DNA in one of the following ways:
   - 5±3°C for up to 24 hours.
   - Below –18°C for long-term storage.
Product description

The MicroSEQ™ Salmonella spp. Detection Kit detects Salmonella spp. simply, reliably, and rapidly in food samples using a lyophilized reagent format. The assay uses the polymerase chain reaction (PCR) to amplify a unique microorganism-specific DNA target sequence and a TaqMan® probe to detect the amplified sequence.

The MicroSEQ™ assay beads contain all the components necessary for the real-time PCR reaction: Salmonella spp.-specific probe and primers, enzyme, and other buffer components. The assay beads also contain an internal positive control (IPC) probe, primers, and template, to monitor for PCR inhibition. Pathogen Detection Negative Control is included in the kit. Unknown samples and positive control samples are provided by the investigator.

RapidFinder™ Express Software provides step-by-step instructions to set up the real-time PCR assays followed by automated data analysis. Online help is provided within the software. The software is designed for use on the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument.
Materials for PCR detection

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.

Table 4  MicroSEQ™ Salmonella spp. Detection Kit [96 reactions; Cat. No. 4403930]

<table>
<thead>
<tr>
<th>Contents</th>
<th>Amount</th>
<th>Cap color</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> spp. Assay Beads, 8-tube strips</td>
<td>12 strips (96 tubes)</td>
<td>Green (rack)</td>
<td>5±3°C Protect from light and moisture.[1]</td>
</tr>
<tr>
<td></td>
<td>1 rack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MicroAmp™ Optical 8-Cap Strips</td>
<td>12 strips (96 caps)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Pathogen Detection Negative Control[2]</td>
<td>1.5 mL</td>
<td>Red</td>
<td>5±3°C</td>
</tr>
</tbody>
</table>

[1] Excessive exposure to light may affect the fluorescent probes. To protect the beads from moisture, do not remove the desiccant from the pouch, and seal the pouch tightly each time you remove assay bead strips.

[2] The Pathogen Detection Negative Control is included in a separate box and may be shipped separately.

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
</table>
| Applied Biosystems™ 7500 Fast Real-Time PCR Instrument | A30304 (desktop)  
A30299 (laptop)  
Contact your local sales representative. |
| RapidFinder™ Express Software Version 1.1 or later | Download the latest version at www.thermofisher.com/us/en/home/technical-resources/software-downloads/rapidfinder-express-software.html |
| 7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips | A29252 |
| MicroAmp™ 96-Well Base                          | N8010531 |
| MicroAmp™ Cap Installing Tool                  | 4330015 |
| MicroAmp™ Multi-removal Tool                    | 4313950 |
| Benchtop microcentrifuge with 8-tube strip adapter or Plate centrifuge | MLS |
| Laboratory mixer (Vortex mixer or equivalent)  | MLS |
### Workflow

**Create or edit a run file in RapidFinder™ Express Software**

**Prepare the assay beads**

**Set up the PCR reactions**

**Load and balance the instrument, then run the reactions**

**View results and data analysis**

---

**Table**

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pipettors:</strong></td>
<td>MLS</td>
</tr>
<tr>
<td>• Positive-displacement</td>
<td></td>
</tr>
<tr>
<td>• Air-displacement</td>
<td></td>
</tr>
<tr>
<td>• Multichannel</td>
<td></td>
</tr>
<tr>
<td><strong>Consumables</strong></td>
<td></td>
</tr>
<tr>
<td>Aerosol-resistant pipette tips</td>
<td>MLS</td>
</tr>
<tr>
<td>Disposable gloves</td>
<td>MLS</td>
</tr>
<tr>
<td>MicroAmp™ Fast 8-Tube Strip, 0.1-mL[^1]</td>
<td>4358293</td>
</tr>
<tr>
<td>MicroAmp™ Optical 8-Cap Strip, 300 strips[^1]</td>
<td>4323032</td>
</tr>
<tr>
<td><strong>Reagents</strong></td>
<td></td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>AM9938</td>
</tr>
</tbody>
</table>

[^1]: Required to evenly distribute the clamping load applied to the tube strips during PCR processing. Do not use other tube strips, which could result in crushed tubes.
Important procedural guidelines for PCR

Software

RapidFinder™ Express Software determines the Run Layout (plate layout) during creation of the run file, therefore it must be set up before distributing DNA samples to the assay beads.

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

Sample handling

- If DNA samples were stored before PCR, thaw (if necessary), vortex, and centrifuge at 1,000–2,000 × g for approximately 1 minute, to remove any condensation from the adhesive film before opening the plate (to avoid cross contamination).
- Use a new pipette tip for each sample.
- If you mix the assay beads with the DNA samples by pipetting up and down, keep the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination.
- Follow the recommendations in “Good laboratory practices for PCR” on page 30.

Avoid fat layer and particulates after sample lysis (collection of DNA sample for PCR)

<table>
<thead>
<tr>
<th>If you see this in the Elution Plate...</th>
<th>Do this...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil droplets as a top layer</td>
<td>After lysis, food samples with high fat or oil content can form a top layer containing fat and debris over the aqueous phase containing the DNA. Collect the DNA sample for PCR from the clear middle phase, avoiding the top layer and bottom pellet (see figure).</td>
</tr>
</tbody>
</table>
| Magnetic Particles                      | 1. Place the Elution Plate on a 96-well magnetic ring stand for at least 1 minute.  
   2. Collect the eluate for PCR while the Elution Plate remains on the magnetic stand.  
   Avoid touching the Magnetic Particles. |
| Particulate residue from food sample    | If the particulate residue is not removed using a 96-well magnetic ring stand:  
   1. Centrifuge the Elution Plates at about 4000 × g for about 30 seconds in a plate centrifuge.  
   2. Avoid the particulate residue, and collect eluate for PCR. |

Figure 1 High-fat samples: Collect sample from middle phase after lysis.
**MicroAmp™ tube strips**

- Follow these instructions to ensure proper storage of the tube strips:
  - Cut the storage pouch at the notch above the resealable strip.
  - Always reseal the storage pouch with desiccant, and replace at 5±3°C.
- 8-tube strips can be cut apart with scissors.
  If necessary, trim any remaining connector material from the cut to allow a better fit against adjacent tubes in the 7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips.
- MicroAmp™ Tube Strips are labeled 1–8 on the side of the tubes, to orient tube strips during handling.

![Figure 2 MicroAmp™ Tube Strip labeling](image)

The tube strip is shown with tinted dome caps, as shipped. For PCR, replace the dome caps with the optical cap strips provided in the kit.

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

- Seal the tubes with the transparent, optical cap strips provided in the kit. Do not use colored caps or tubes for real-time PCR reactions, because they may affect dye-signal readings during real-time PCR.
- Always use intact 8-cap strips, even if empty tubes have been added next to reaction tubes.
- Use the MicroAmp™ 96-Well Base and the MicroAmp™ Cap Installing Tool to seal the assay tubes with the optical cap strips. This avoids collapsing, bending, or misaligning the tubes.
  Confirm that the strips are straight and that each tube is in line with the adjacent tube.
- Use a plate adapter for vortexing the tube strips, or hold the strips in the MicroAmp™ 96-Well Base while vortexing.

**Create or edit a run file in RapidFinder™ Express Software**

On the main page of the RapidFinder™ Express Software, select Create/Edit a Run File, and select the target pathogen, number of samples, replicates, and positive and negative controls for each target at the prompts.

The software determines the sample layout based on the information entered, and creates a run file.

**Prepare the assay beads**

Follow the plate layout determined by the RapidFinder™ Express Software.
1. Transfer the appropriate number of individual tubes or 8-tube strips from the storage pouch to a 96-well base at room temperature (23±5°C).

2. If required by the plate layout, place empty MicroAmp™ Fast 8-Tube Strips (or partial strips) to balance the tray when the assay tubes are placed in the instrument later.

## Set up the PCR reactions

If you are using RapidFinder™ Express Software v1.1, step-by-step instructions are available through Pipette Samples on the main page.

1. If necessary, thaw samples and controls completely, and mix each sample or control thoroughly.
   - If the Elution Plate contains oil droplets, magnetic particles, or food particulate residue, see “Avoid fat layer and particulates after sample lysis (collection of DNA sample for PCR)” on page 20.
   - If the DNA samples have been stored, see “Sample handling” on page 20.

2. Following the layout determined by RapidFinder™ Express Software, add 30 µL of sample or control to each assay bead at room temperature (23±5°C), and mix by gently pipetting up and down a few times. Beads dissolve in 1–5 seconds.
   - Alternatively, vortex the assay tubes after they are capped in the final step.

3. Seal the tubes with the transparent, optical cap strips provided in the kit.

4. Make sure that the reactions are thoroughly mixed: if reactions were not previously mixed during the pipetting step, vortex to mix.

5. Make sure that the reagents are at the bottom of tubes: briefly centrifuge the tube strips at 200–600 × g for about 20 seconds.

## Load and run the reactions

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

1. Transfer the tubes to the instrument in the same configuration as the run layout.
   - Use the 7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips in the instrument.
   - Be sure to load empty tube strips as directed by the software (Figure 3).

2. Close the tray to the instrument, and follow the RapidFinder™ Express Software prompts to start the run.
Figure 3  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.

Follow the RapidFinder™ Express Software prompts for "Investigating Warning Results or Failed Runs in the SDS Software."

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

1. From **View Results** in the RapidFinder™ Express Software, select and open the run file, and then click **View in SDS**.

2. Select **File > Save As**, and save the run file under a new name.
In the context of AOAC validation, enriched cultures with positive PCR results were tested further by cultural confirmation using the appropriate reference method for the sample matrix (see “AOAC Performance Tested Methods® Certification” on page 30).
## Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial pellet is difficult to avoid during removal of supernatant</td>
<td>The sample was left unattended before removal of the supernatant, causing dissipation of the bacterial pellet.</td>
<td>Remove the supernatant immediately following centrifugation.</td>
</tr>
<tr>
<td></td>
<td>The size of the bacterial pellet is very small and difficult to see.</td>
<td>Remove the supernatant carefully, leaving behind up to 50 µL of supernatant, to avoid aspiration of the pellet.</td>
</tr>
<tr>
<td>Bacterial pellet is difficult to resuspend during lysis</td>
<td>Pellet is too hard.</td>
<td>Ensure maximum resuspension of the pellet in the Lysis Buffer or Proteinase K Buffer Mix before proceeding. Transfer the entire contents, including the incompletely resuspended pellet (if any) to the Lysis Plate. Follow the PrepSEQ™ Preclarification Protocol (&quot;For large sample pellets: perform the preclarification protocol&quot; on page 14).</td>
</tr>
<tr>
<td>Inhibition of downstream PCR, indicated by nondetection of IPC reaction</td>
<td>Magnetic Particles were in the Elution Plate.</td>
<td>Avoid disturbing the Magnetic Particles during transfer of eluted DNA to the lyophilized assay. Avoid transfer of Magnetic Particles using one of the following methods <em>(optional)</em>: • Place the Elution Plate on the 96-Well Magnetic Ring Stand during transfer of eluted DNA sample to the lyophilized assay. • Spin the plate at maximum speed in a plate centrifuge for the equivalent of approximately 4,000 × g for approximately 30 seconds, to pellet the Magnetic Particles to the bottom of the plate.</td>
</tr>
<tr>
<td></td>
<td>Elution Plate contains incompletely removed particulate residue from the food sample.</td>
<td>Avoid residue during transfer of eluted DNA to the lyophilized assay. <em>(Optional)</em> Spin the plate at maximum speed in a plate centrifuge for the equivalent of approximately 4,000 × g for approximately 30 seconds, to pellet the food residue to the bottom of the plate.</td>
</tr>
<tr>
<td>Observation</td>
<td>Possible cause</td>
<td>Recommended action</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Inhibition of downstream PCR, indicated by nondetection of IPC reaction</td>
<td>For samples of enriched culture that were centrifuged before lysis, the removal of sample supernatant before addition of lysis buffer was incomplete.</td>
<td>Ensure maximal removal of the supernatant without disturbing the bacterial pellet.</td>
</tr>
<tr>
<td>In positive control wells, no IPC signal is detected, but target-specific signal is detected</td>
<td>A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.</td>
<td>No action is required. The result is considered positive.</td>
</tr>
<tr>
<td>In positive control wells, no target-specific signal is detected</td>
<td>Positive control was omitted (pipetting error).</td>
<td>Repeat the assay. Make sure to pipet the positive control into all positive control wells.</td>
</tr>
<tr>
<td>In negative control wells, no IPC signal is detected, but a target-specific signal is detected</td>
<td>Carryover contamination caused target signal in negative control wells. Additionally, no IPC signal in negative control wells can be caused by:</td>
<td>To correct carryover contamination, repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.</td>
</tr>
<tr>
<td></td>
<td>• A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.</td>
<td>To determine whether IPC amplification is a problem, examine unknown wells for an IPC signal. If an IPC signal is present, IPC amplification is not a problem.</td>
</tr>
<tr>
<td>In negative extraction control wells, target-specific signal is detected</td>
<td>Carryover contamination occurred.</td>
<td>1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. If the negative extraction control continues to show contamination, repeat the assay using a new kit.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If the negative extraction control continues to show contamination, contact Technical Support.</td>
</tr>
<tr>
<td>In unknown wells, no IPC or target-specific signal is detected</td>
<td>Inhibition of PCR occurred.</td>
<td>Dilute the sample 1:5 with Nuclease-free Water to dilute PCR inhibitors, and repeat the assay. If PCR remains inhibited, repeat the sample preparation. Refer to other troubleshooting suggestions for removal of Magnetic Particles or particulate residue from the DNA sample.</td>
</tr>
<tr>
<td>In unknown wells, no IPC signal is detected, but target-specific signal is detected</td>
<td>A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.</td>
<td>No action is required. The result is considered positive.</td>
</tr>
<tr>
<td><strong>Observation</strong></td>
<td><strong>Possible cause</strong></td>
<td><strong>Recommended action</strong></td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------</td>
<td>------------------------</td>
</tr>
</tbody>
</table>
| Multicomponent plot signals for FAM™, VIC™, and ROX™ detectors increase/decrease during cycles 1–15, but the amplification curve and result are not affected [this observation applies to View in SDS mode]. | Incomplete mixing and dissolution of the lyophilized bead with sample or control. | After addition of 30 µL of sample or Pathogen Negative Control to the bead and capping the tubes:  
1. Vortex strips at high speed for about 10 seconds, and centrifuge the strips at 200–600 × g for about 10 seconds.  
2. Vortex the strips again on high speed for about 10 seconds, and centrifuge the strips at 200–600 × g for about 1 minute.  
Ensure that all liquid is at the bottom of the tubes and the beads are fully dissolved before proceeding. |
| Replicate results for a sample are inconsistent. | All replicate wells for a sample do not have the same result. | If more than two replicates yield the same result (for example, 2 of 3 replicates are negative, but 1 replicate is positive), refer to your laboratory protocol to determine whether to repeat the assay using fresh samples and reagents.  
If only 2 replicates were run and the results are not consistent, repeat the assay using fresh samples and reagents. |
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| Amplicon contamination.     | • Contamination was introduced into the PCR clean area from post-amplification reaction tubes that were either opened in the clean area or brought into the PCR clean area from contaminated gloves or solutions.  
• Contamination was introduced into the real-time PCR instrument from crushed and broken PCR reaction tubes. | To confirm amplicon contamination, perform the following experiment:  
Prepare negative control samples using at least one 8-tube strip of MicroSEQ™ Assay Beads.  
1. Divide the assay beads into two sets.  
   a. To the first set of assay beads, add 30 μL of Nuclease-free Water.  
   b. To the second set of assay beads, add 29 μL of Nuclease-free Water plus 1 μL of 1 U/μL Uracil DNA Glycosylase [Cat. No. 18054-015].  
2. Run samples on the 7500 Fast Real-Time PCR Instrument using SDS software and select Fast 7500 run mode.  
3. Under the instrument tab:  
   • Select Add Step to stage 1 of the PCR cycle that consists of 10 minutes at 50°C.  
   • Extend the 95°C step from 20 seconds to 10 minutes.  
   Amplicon contamination is indicated by target-specific signal in the –UNG samples and no target-specific signal in +UNG samples.  
If the instrument block was contaminated, consult the Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve (Pub. No. 4347825) and/or contact a service representative to clean the instrument. |
Supplemental information

Sensitivity

The sensitivity of the assay in real culture samples depends on the quality of the sample preparation method that is used. The AOAC Performance Tested Methods\textsuperscript{SM} workflow described in this user guide allows you to detect 1 to 3 colony-forming units (CFU) in 25 grams of food or environmental swab and sponge samples. Following the workflow described in this user guide, the limit of detection is $10^3$ cfu/mL (not part of AOAC validation study).

Specificity

The MicroSEQ\textsuperscript{TM} Salmonella spp. Detection Kit can detect all *Salmonella enterica* serovars tested and did not detect any non-*Salmonella* species tested. The genus *Salmonella* consists of the two species *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* incorporates the most important clinical serovars for humans. The method does not allow detection of *Salmonella bongori*.

Operational conditions

**Table 5** Magnetic Particle Processor operation conditions (for indoor use only)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Acceptable range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>10–40°C</td>
</tr>
<tr>
<td>Humidity</td>
<td>Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C</td>
</tr>
</tbody>
</table>

**Table 6** Applied Biosystems\textsuperscript{TM} 7500 Fast Real-Time PCR Instrument operation conditions (for indoor use only)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Acceptable range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>10–40°C</td>
</tr>
<tr>
<td>Humidity</td>
<td>Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C</td>
</tr>
<tr>
<td>Altitude</td>
<td>Not exceeding 2,000 m (6,500 ft) above sea level</td>
</tr>
</tbody>
</table>
AOAC Performance Tested Methods™ Certification

Go to thermofisher.com/foodsafety for a list of workflows for detection of Salmonella spp.

Table 7  Performance Tested Methods™ Certification of the workflow

<table>
<thead>
<tr>
<th>Certification</th>
</tr>
</thead>
<tbody>
<tr>
<td>![AOAC Certification logo]</td>
</tr>
</tbody>
</table>

The detection of Salmonella spp. using PrepSEQ™ kits and MicroSEQ™ Salmonella spp. Detection Kit has earned the AOAC Performance Tested Methods™ Certification from the AOAC Research Institute. The certified workflow described in this user guide includes:

- Enrichment media: Buffered Peptone Water (with skim milk powder and Brilliant Green Dye Solution for chocolate matrices)
- PrepSEQ™ Nucleic Acid Extraction Kit
- MicroSEQ™ Salmonella spp. Detection Kit
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software Version 1.1 or later
- Confirmation testing of positive samples

<table>
<thead>
<tr>
<th>Reference method</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>EN ISO 6579:2017</td>
<td>Food: raw ground beef, raw chicken wings, raw shrimp, cantaloupe, brie, dry infant formula, chocolate, 25 g dry pet food, shell eggs, black pepper</td>
</tr>
<tr>
<td>USFDA BAM, Chapter 5</td>
<td>Food: peanut butter Environmental samples: stainless steel, sealed concrete, plastic, ceramic tile, rubber</td>
</tr>
</tbody>
</table>

Good laboratory practices for PCR

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

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Do not open reaction tubes after PCR.
- Do not autoclave reaction tubes after PCR.
- Clean lab benches and equipment periodically with 10% bleach solution or DNAZap™ Solutions (Cat. No. AM9890).

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.
Chemical safety

**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).

- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.

- Handle chemical wastes in a fume hood.

- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)

- After emptying a waste container, seal it with the cap provided.

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.

- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

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

---

**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! 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.

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.

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

<table>
<thead>
<tr>
<th>Document</th>
<th>Publication number</th>
</tr>
</thead>
<tbody>
<tr>
<td>RapidFinder™ Express Software Quick Reference</td>
<td>4480999</td>
</tr>
<tr>
<td>Thermo Scientific™ KingFisher™ Flex User Manual</td>
<td>N07669</td>
</tr>
<tr>
<td>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide</td>
<td>4378657</td>
</tr>
<tr>
<td>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide</td>
<td>4387777</td>
</tr>
</tbody>
</table>
References

