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

USER GUIDE

Using spin-column–based DNA isolation methods

for use with:
PrepSEQ™ Rapid Spin Sample Preparation Kit
PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean
MicroSEQ™ *Salmonella* spp. Detection Kit
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
RapidFinder™ Express Software

Publication Number 4412848
Revision G

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 031001

For testing of Food and Environmental samples only.

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Revision history: Revision history of Pub. no. 4412848

Revision	Date	Description
G	26 June 2017	• Added information for analysis of 375-g pet food samples. • Clarified that enriched cultures should be mixed before transferring sample to the spin column. • Updated logos and legal statements per corporate guidelines.
F	November 2014	This user guide is now a complete AOAC™ *Performance Tested Methods*℠ workflow that covers enrichment, DNA isolation, and real-time PCR detection. • Added instructions for using the MicroSEQ™ *Salmonella* spp. Detection Kit with the DNA that was isolated using the PrepSEQ™ Rapid Spin Sample Preparation Kit. • Removed the AFNOR™ NF VALIDATION™ information and instructions to a separate user guide (see Pub. no. MAN0010012). • Reorganized some content for clarity and to match current style guides. • Updated company name to Thermo Fisher Scientific.
User guide for using the PrepSEQ™ Rapid Spin Sample Preparation Kit to isolate DNA from *Salmonella* spp.:		
E	November 2013	• Added details about AOAC™ *Performance Tested Methods*℠ certification. • Updated number formats (for example, time, temperature, and centrifugation speeds). • Added instructions for collecting and enriching environmental samples for AOAC™ certification. • Updated document template with associated updates to the limited license information, warranty information, trademark statement, and safety statements.
D	October 2011	Added AFNOR™ seal and text, including an update of number formatting (for example, temperature and time range).
C	September 2009	Baseline for revision history.

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Contents

1 Overview

IMPORTANT! Before using the products described in this guide, read and understand the information in the "Safety" appendix in this document.

This guide describes the following AOAC™ *Performance Tested Methods*℠ workflow for detection of *Salmonella* spp. in food and environmental samples.

1. Enrichment of samples in Buffered Peptone Water (BPW).
   - 25-g food samples or environmental samples
   - 375-g dry pet food samples
2. Preparation of PCR-ready DNA using the PrepSEQ™ Rapid Spin Sample Preparation Kit.

   For some foods with a high-lipid content, such as infant formula, soft cheese, and chicken wing samples, use thePrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean.
3. Real-time PCR detection of *Salmonella* spp. DNA in the DNA sample using the MicroSEQ™ *Salmonella* spp. Detection Kit and RapidFinder™ Express Software on the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument.

   Note: RapidFinder™ Express Software Version 1.2 or higher is required for analysis of 375-g pet food samples.
4. Confirmation testing of positive samples by an independent method.

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.

See "AOAC™ *Performance Tested Methods*℠ Certification" on page 26 for detailed information.

Visit **thermofisher.com/foodsafety** for a complete list of workflows for detection of *Salmonella* spp. (Pub. no. MAN0009417).

2 Enrich food or environmental samples

Required materials

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

Item	Source
Buffered Peptone Water (BPW)	Oxoid™ CM1049, DB1049, DF1049, or equivalent
Skim milk powder (for chocolate samples only)	MLS
Brilliant Green Dye Solution (for chocolate samples only)	MLS
Dey Engley (D/E) Neutralizing Broth (for environmental swabs or sponges)	BD # 281910 or equivalent
Swabs, cotton	MLS
Sterile 15-mL conical tubes (for environmental swab samples)	MLS
Homogenizer (Stomacher™ 400 Laboratory Blender or equivalent)	Thermo Scientific™ DB5000A, or equivalent
Homogenizer bag, as required by sample type:	
With sponge, 4.5" × 9" (Whirl-Pak™ Speci-Sponge Environmental Sampling Bag, or equivalent)	Nasco # B01245WA or equivalent
With mesh, 6" × 9", 24 oz (Whirl-Pak™ Filter Bag for Homogenizer Blender, or equivalent)	Nasco # B01348WA or equivalent
No mesh, 6" × 9", 24 oz (Whirl-Pak™ Write-on Bag, or equivalent)	Nasco # B01196WA or equivalent
For 375-g samples: Homogenizer bag, 15" × 20", 184 oz. (Whirl-Pak™ Bag for Homogenizer Blenders, or equivalent)	Nasco # B01447WA or equivalent

Enrich 25-g food samples or environmental 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.

Sample type [1]	Method
Food	Add 225 mL of BPW (+ skim milk powder for chocolate samples) to 25 g (or 25 mL) of sample.
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 26

3. Mix the sample with BPW as described in the following table:
   A filtered bag may be used for enrichment of samples with particulates.

Sample type	Method
Food: • Coarse food types, such as meat, poultry, and seafood[1] • Eggs	Process for 1–2 minutes in a homogenizer (Stomacher™ 400 Laboratory Blender with speed setting **Norm**, or equivalent).
Food: Liquids or powdered	Shake the bag at least 25 times to achieve a homogeneous suspension.
Environmental swab	Twirl the swab for 90±30 seconds.
Environmental sponge	Use one of the following methods: • Use a nonfiltered, nonmesh enrichment bag and homogenize for about 1 minute (Stomacher™ 400 Laboratory Blender with speed setting **Norm**, or equivalent) • Hand squeeze for about 1 minute.

[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.

Enrich 375-g pet food samples

1. Prepare Buffered Peptone Water (BPW) according to the manufacturer's instructions.
2. For each 375-g pet food sample, prewarm 3.375 L of BPW at 37±1°C overnight (minimum 8 hours).
3. Combine 3.375 L of prewarmed BPW with 375 g of dry pet food in a homogenizer bag, and mix thoroughly.
4. Incubate at 37±1°C under static conditions for 20–24 hours.

3 Isolate DNA using the PrepSEQ™ Rapid Spin Sample Preparation Kit

Product description

The PrepSEQ™ Rapid Spin Sample Preparation Kit enables rapid preparation of PCR-ready bacterial DNA from broth cultures. In a simple spin step, the media inside the spin column removes PCR inhibitors from the sample, allowing the bacteria to pass through into the sample collection tube for DNA extraction.

For samples with a high lipid content, the PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean is recommended. The procedure includes an additional step for separation of the aqueous phase from lipid. Both procedures are described in this chapter.

Kit contents and storage

Table 2 PrepSEQ™ Rapid Spin Sample Preparation Kit (100 reactions, Cat. no. 4407760)

Components	Quantity or volume	Storage[1]
Spin columns	100	Room temperature (23±5°C)
Microcentrifuge tubes, 1.5 mL	100	
Lysis Buffer, 1 bottle	5 mL	5±3°C

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

Table 3 PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean (100 reactions, Cat. no. 4413269)

Components	Quantity or volume	Storage[1]
Spin columns	100	Room temperature (23±5°C)
Microcentrifuge tubes, 1.5 mL	2 × 100	
Lysis Buffer, 1 bottle	5 mL	5±3°C

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

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

Required materials not included with the kit

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

Item	Source
Equipment	
Block heater, 95°C	MLS
Rack for 1.5-mL tubes	MLS
Benchtop microcentrifuge	Eppendorf 5415 D or equivalent
Laboratory mixer, Vortex or equivalent	MLS
Pipettors: • Positive-displacement • Air-displacement	MLS
Additional consumables	
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS
Reagents	
Nuclease-free Water	Cat. no. AM9938

Workflow

Filter the enriched sample through the spin column

Insert a spin column into a labeled tube.

▼

Load 750 µL of the enriched sample onto the spin column and cap the column.

▼

Microcentrifuge the tube at 12,000–16,000 × *g* for about 3 minutes.

▼

Discard the used spin column. Then, aspirate and discard the supernatant.

▼

Lyse the sample

Add 50 µL of Lysis Buffer to the pellet, and pipet up and down or vortex to mix.

▼

Rapid Spin Extra Clean protocol only: transfer the Lysis Buffer mixture to a clean 1.5-mL tube, avoiding the fat.

▼

Cap the tube, then incubate at 97±2°C for 12±2 minutes.

▼

Allow the sample to cool for about 2 minutes at room temperature (23±5°C).

▼

Microcentrifuge the tube at 12,000–16,000 × *g* for about 1 minute.

▼

Add 250 µL of Nuclease-free Water, and mix well.

▼

Microcentrifuge the tube at 12,000–16,000 × *g* for 1–2 minutes.

▼

Store DNA or proceed to PCR

Important procedural guidelines for DNA preparation

PCR-clean water

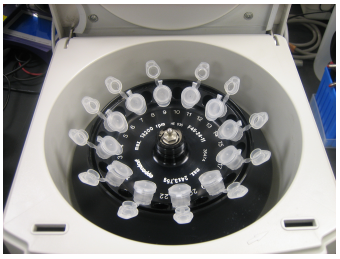
Use Nuclease-Free Water (Cat. no. AM9938) for all procedures described in this protocol that require water. Nuclease-Free Water is considered "PCR-clean" water. However, autoclaved water should not be considered PCR-clean water.

Mix the enriched culture before sample collection

After incubation, gently mix the enriched culture in the homogenizer bag to distribute microbes within the mixture. If necessary, allow the bag to settle for 5–15 minutes before sample collection, to minimize transfer of particulates.

Position of the spin column/tube assembly in the microcentrifuge

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



Incorrect position of tube caps

Correct position of tube caps

For high-fat samples: remove fat layer before lysis

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

Type of fat layer	Fat layer and supernatant removal
Liquid	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.

Filter 750 µL of enriched culture through the spin column

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

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

2. Microcentrifuge the spin column assembly at 12,000–16,000 × *g* for about 3 minutes.

   Follow “Position of the spin column/tube assembly in the microcentrifuge“ on page 12.

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

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

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

   (Optional) If necessary, follow "For high-fat samples: remove fat layer before lysis" on page 12

Lyse the sample

1. Add 50 µL of Lysis Buffer to the pellet, and pipet up and down or vortex until the pellet is well dispersed in the Lysis Buffer mix.

2. (*Optional*) **Rapid Spin Extra Clean protocol (for samples with high lipid content)**: transfer the mixture to a clean 1.5-mL tube, avoiding residual fat.

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

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

3. Cap the tube, then incubate at 97±2°C for 12±2 minutes.

4. Allow the sample to cool for about 2 minutes at room temperature (23±5°C).

5. Microcentrifuge the tube at 12,000–16,000 × *g* for about 1 minute to collect the contents at the bottom of the tube.

6. Add 250 µL of Nuclease-free Water, and mix thoroughly.

7. Microcentrifuge the tube at 12,000–16,000 × *g* for 1–2 minutes to pellet any remaining particulate material.

   The microbial DNA is in the supernatant.

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

- At 5±3°C for up to 24 hours.
- Below –18°C for up to 1 year.

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

4 PCR with the MicroSEQ™ *Salmonella* spp. Detection Kit and RapidFinder™ Express Software

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.

Kit contents and storage

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

Component	Description	Amount	Cap color	Storage
MicroSEQ™ *Salmonella* spp. Detection Kit	*Salmonella* spp. Assay Beads, 8-tube strips	12 strips (96 tubes) 1 rack	Green (rack)	5±3°C Protect from light and moisture.[1]
	MicroAmp™ Optical 8-Cap Strips	12 strips (96 caps)	N/A	
Pathogen Detection Negative Control[2]	Pathogen Detection Negative Control	1.5 mL	Red	5±3°C

[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.

Note: Kit parts may ship separately, depending on the configuration ordered and storage conditions.

Required materials not included with the kit

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

Item	Source
Instruments and equipment	
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument	Cat. no. A30304 (desktop) Cat. no. A30299 (laptop) Contact your local sales representative.
RapidFinder™ Express Software Version 1.1 or higher; Version 1.2 or higher required for 375-g pet food samples[1]	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	Cat. no. 4403809
MicroAmp™ 96-Well Base	Cat. no. N8010531
MicroAmp™ Cap Installing Tool	Cat. no. 4330015
MicroAmp™ Multi-removal Tool	Cat. no. 4313950
Benchtop microcentrifuge with 8-tube strip adapter *or* Plate centrifuge	MLS
Laboratory mixer (Vortex mixer or equivalent)	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
Consumables	
Aerosol-resistant pipette tips	MLS
Disposable gloves	MLS
MicroAmp™ Fast 8-Tube Strip, 0.1-mL[2]	Cat. no. 4358293
MicroAmp™ Optical 8-Cap Strip, 300 strips[2]	Cat. no. 4323032
Reagents	
Nuclease-Free Water	Cat. no. AM9938

[1] Analysis of pet food samples requires custom analysis settings; contact Technical Support. Versions 1.1 and 1.2 require use of the Windows™ XP operating system. Version 2 requires the Windows™ 7 operating system only.

[2] 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.

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

Important procedural guidelines

Software

The plate layout is determined during creation of the run file in RapidFinder™ Express Software, therefore the software must be set up before distributing DNA samples to the assay beads.

IMPORTANT! Analysis of 375-g pet food samples requires RapidFinder™ Express Software Version 1.2 or higher and custom analysis settings (contact Technical Support).

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

Sample handling

- If DNA samples have been stored or the pellet has dispersed, thaw the samples (if necessary), vortex, and centrifuge at 12,000–16,000 × *g* for 1–2 minutes. This step will avoid cross-contamination and exclude particulate matter from the PCR.
- Use a new pipette tip for each sample.
- If you mix the assay beads with the DNA samples by pipetting up and down, keep the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination.
- Follow the recommendations in "Good laboratory practices for PCR" on page 26.

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

After lysis, food samples with high fat or oil content can form a top layer containing fat and debris over the aqueous phase containing the DNA. Collect the DNA sample for PCR from the clear middle phase, avoiding the top layer and bottom pellet.

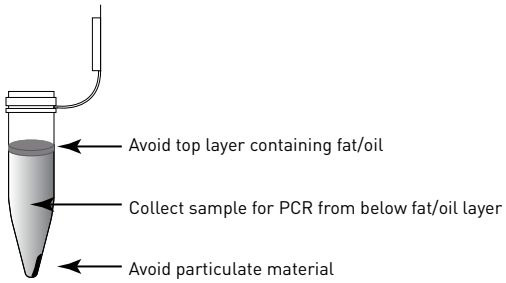


Figure 1 High-fat samples: collect sample from middle phase after lysis.

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.

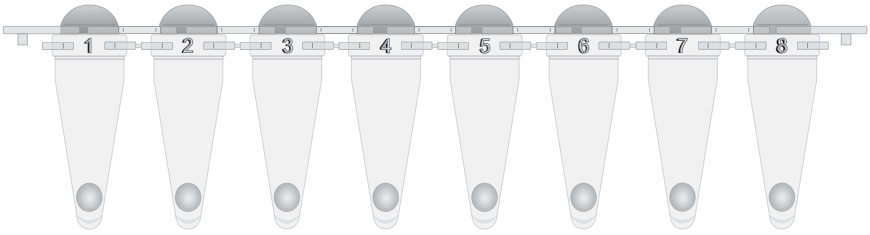


Figure 2 MicroAmp™ Tube Strip labeling
The tube strip is shown with tinted dome caps, as shipped. For PCR, replace the dome caps with the optical cap strips provided in the kit.

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 DNA samples have been stored or the pellet has dispersed, see"Sample handling" on page 16 .

   If the sample contains oil droplets or food particulate residue, see"For high-fat samples after lysis: collection of DNA sample for PCR" on page 17.

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 using a centrifuge with a plate adapter or a benchtop microcentrifuge with an 8-strip PCR tube adapter.

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.

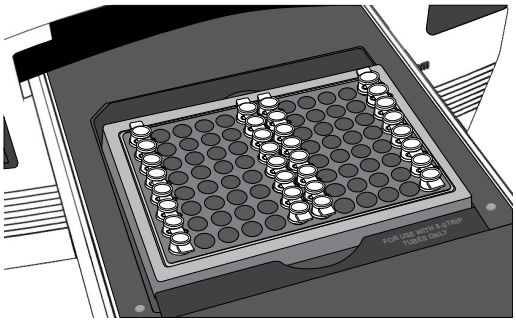


Figure 3 7500 Fast instrument tube layout

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

In the RapidFinder™ Express Software, select **View Results** on the main page, select the appropriate run file, and follow the prompts to view results.

Data analysis is automated by the software.

If necessary, investigate results in SDS Software

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.

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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.

5 Recommended confirmation methods

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 26).

A Troubleshooting

Observation	Possible cause	Recommended action
Bacterial pellet is difficult to avoid during removal of supernatant	The sample was left unattended before removal of the supernatant, causing dissipation of the bacterial pellet.	Remove the supernatant immediately following centrifugation.
	The size of the bacterial pellet is very small and difficult to see.	Remove the supernatant carefully, leaving behind up to 50 µL of supernatant, to avoid aspiration of the pellet.
The PCR was inhibited, as indicated by non-detection of the IPC reaction.	Removal of the supernatant was insufficient before addition of Lysis Buffer.	Dilute the sample 1:5 or 1:10 with Nuclease-free Water to dilute PCR inhibitors. If PCR remains inhibited, repeat the sample preparation.
	Filtrate from the spin column is in the sample.	Centrifuge the sample to separate the filter particulates before transferring sample to the PCR .
	Excess fat was not removed during aspiration of the supernatant.	Apply PrepSEQ™ Rapid Spin extra clean protocol.
	The sample matrix is associated with PCR-inhibitory components.	Pre-wash the bacterial pellet before loading the Rapid Spin column: 1. Transfer 750 µL of sample to a clean microcentrifuge tube. 2. Centrifuge at 12,000–16,000 × *g* for about 3 min. 3. Discard supernatant. 4. Resuspend pellet in 650 µL of sterile distilled water. 5. Load the resuspended sample onto the spin column.
In positive control wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.	No action is required. The result is considered positive.
In positive control wells, no target-specific signal is detected.	Positive control was omitted (pipetting error).	Repeat the assay. Make sure to pipet the positive control into all positive control wells.
In negative control wells, no IPC signal is detected, but a target-specific signal is detected	Carryover contamination caused target signal in negative control wells.	To correct carryover contamination, repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.

Observation	Possible cause	Recommended action
In negative control wells, no IPC signal is detected, but a target-specific signal is detected	Additionally, no IPC signal in negative control wells can be caused by: • A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA. • A problem occurred with IPC amplification.	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.
In negative control wells, target-specific signal is detected	Carryover contamination occurred.	1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 2. If the negative control continues to show contamination, repeat the assay using a new kit. 3. If the negative control continues to show contamination, contact Technical Support.
In unknown wells, no IPC or target-specific signal is detected.	Inhibition of PCR occurred.	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 particulates from the DNA sample.
In unknown wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.	No action is required. The result is considered positive.
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.

Observation	Possible cause	Recommended action
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.
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 *7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide* (Pub. no. 4347825) and/or contact a service representative to clean the instrument.

B

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*℠ 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™ *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

The Applied Biosystems™ 7500 Fast Real-Time PCR Instrument is for indoor use only and for altitudes not exceeding 2,000 m (6,500 ft) above sea level.

Condition	Acceptable range
Temperature	15–30°C Maximum change of less than 15°C per 24 hours
Humidity	20–80% relative humidity, noncondensing

AOAC™ *Performance Tested Methods*℠ Certification

Visit **thermofisher.com/foodsafety** for a list of workflows for detection of *Salmonella* spp.

Table 5 *Performance Tested Methods*℠ Certification of the workflow

Certification
PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 031001

The detection of *Salmonella* spp. using PrepSEQ™ kits and MicroSEQ™ *Salmonella* spp. Detection Kit has earned the *Performance Tested Methods*℠ Certification from the AOAC™ Research Institute. The validated 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™ Rapid Spin Sample Preparation Kit
- MicroSEQ™ *Salmonella* spp. Detection Kit
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software Version 1.1 or higher; Version 1.2 or higher required for 375-g pet food samples[1]
- Confirmation testing of positive samples

Reference method	Matrix
ISO 6579:2002	**Food**: raw ground beef, raw chicken wings, raw shrimp, cantaloupe, brie, dry infant formula, chocolate, 25 g dry pet food, shell eggs, black pepper
USFDA BAM, Chapter 5	**Food**: peanut butter, 375 g dry pet food **Environmental samples**: stainless steel, sealed concrete, plastic, ceramic tile, rubber

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.

[1] Analysis of pet food samples requires custom analysis settings; contact Technical Support. Versions 1.1 and 1.2 require use of the Windows™ XP operating system. Version 2 requires the Windows™ 7 operating system only.

- 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).

For additional information, refer to ISO 22174:2005.

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, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

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

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Food Safety support

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

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- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

References

ISO. 2002. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. Reference number 6579:2002.

U.S. Food and Drug Administration, Bacteriological Analytical Manual (BAM), Chapter 5; go to **www.fda.gov/food/foodscienceresearch/laboratory methods/ucm2006949.htm** and scroll to *Salmonella*. Accessed 23 Sept. 2013.