Real-Time PCR Detection of *Salmonella* spp. in Meat Samples

USER GUIDE

Using automated DNA isolation and magnetic bead-based technology

for use with:
PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing
KingFisher™ Flex Purification System with 96 Deep-Well Head
MagMAX™ Express-96 Magnetic Particle Processor
MicroSEQ™ Salmonella spp. Detection Kit
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
RapidFinder™ Express Software v2.0 or later

Catalog Numbers 4403930, 4412639, 4428176, 4480466

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Revision E

BY AFNOR CERTIFICATION
NF VALIDATION EN ISO 16140

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For testing of Food and Environmental samples only.

ThermoFisher SCIENTIFIC

Revision history: MAN0018459 E (English)

Revision	Date	Description
E	7 April 2025	The EN ISO references for 7218:2007 and 22174:2005 were updated to reflect the 2024 version with new title—Microbiology of the food chain.
D	8 August 2024	Troubleshooting was added for possible instance of varying morphology of PCR pellets.
C.0	13 July 2020	• The software version was updated for RapidFinder™ Express Software. • Characteristics were added for the 7500 Fast Real-Time PCR Instrument.
B.0	7 August 2020	The legal statement was moved from the front cover to page 2.
A.0	29 January 2019	New document for the *Real-Time PCR Detection of Salmonella spp. in Meat Samples User Guide (Automated DNA Isolation, AFNOR)*. Supersedes Pub. No. 4485954 Rev. D. Additional changes: • The storage temperature of Magnetic Particles was changed from 5±3°C to ambient temperature (15°C to 30°C). • The storage temperatures of other kit components were aligned to the temperatures displayed on the product labels. • The procedural guidelines for PCR were changed to align with current style; information was added about avoiding fat layer or particulates when collecting DNA for PCR.

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

For testing of Food and Environmental samples only.

Contents

1 Overview

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

General overview

This guide describes the following NF VALIDATION™-certified workflow for detection of *Salmonella* spp. in meat samples:

1. Enrichment of 25-g meat samples in Buffered Peptone Water (BPW).
2. Automated preparation of PCR-ready DNA using the PrepSEQ™ Nucleic Acid Extraction Kit and the KingFisher™ Flex Purification System with 96 Deep-Well Head or the MagMAX™ Express-96 Magnetic Particle Processor. Both instruments enable high-throughput sample processing in a 96-well format with minimal handling.
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.
4. Confirmation testing of positive samples by an independent method.

See "NF VALIDATION™ by AFNOR™ Certification" on page 24 for detailed information about the NF VALIDATION™ certification.

Required materials

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

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

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

Materials for enrichment of food samples

Table 1 Equipment, accessories, and consumables

Item	Source
Homogenizer laboratory blender or diluter, one of the following or equivalent:	
• Homogenizer Laboratory Blender • Diluflux™ Pro Automated Gravimetric Dilutor with simple (non-robotic) dispensing arm • Diluflux™ Pro Automated Gravimetric Dilutor with robotic dispensing arm	DB5000A DB4100A DB4150A
Incubator fitted with racks for homogenizer bags	**thermofisher.com**
Sample enrichment bags, one of the following or equivalent:	
• BagFilter™ 400 (400 mL) • BagPage™ 400 (400 mL) • BagLight™ 400 (400 mL) • RollBag™ 1300 (1300 mL)	DB4011A DB4012A DB4013A DB4014A
Reagents	
Buffered Peptone Water (BPW)	DF1049, CM1211B, or equivalent

Materials for DNA Isolation

Table 2 PrepSEQ™ Nucleic Acid Extraction Kit

Contents	Cat. No. 4480466 (100 reactions)	Cat. No. 4428176 (300 reactions)	Storage[1]
Lysis Buffer	2 × 50 mL	6 × 50 mL	15°C to 30°C
Magnetic Particles	2 × 1.5 mL	6 × 1.5 mL	
Binding Solution (Isopropanol)[2]	1 empty bottle	3 empty bottles	
Wash Buffer Concentrate[3]	2 × 26 mL	6 × 26 mL	

Table 2 PrepSEQ Nucleic Acid Extraction Kit *(continued)*

Contents	Cat. No. 4480466 (100 reactions)	Cat. No. 4428176 (300 reactions)	Storage[1]
Elution Buffer	25 mL	3 × 25 mL	15°C to 30°C
Proteinase K (PK) Buffer	50 mL	3 × 50 mL	
Proteinase K, 20 mg/mL	1.25 mL	3 × 1.25 mL	–25°C to –15°C

[1] See the expiration date on the box.
[2] Add ~35 mL of 100% isopropanol to the empty bottle before use.
[3] Add 74 mL of 95% ethanol before use.

Table 3 Magnetic particle processor

Item	Source
KingFisher™ Flex-96 instrument and accessories	
KingFisher™ Flex Purification System with 96 Deep-Well Head	A32681, 96 deep-well plate, or equivalent[1]
KingFisher™ 96 Deep-Well Plate, V-bottom	95040450
KingFisher™ 96 KF microplates (200 µL)	97002540
KingFisher™ Flex 96 heating block	24075420
KingFisher™ 96 tip comb for deep-well magnets	97002534
Finntip™ Filtered Pipette Tips	94052320 or equivalent
MagMAX™ Express-96 instrument and accessories	
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	Contact your local sales representative.
MagMAX™ Express-96 Deep Well Plates	4388476
MagMAX™ Express-96 Standard Plates	4388475
MagMAX™ Express-96 Deep Well Tip Combs	4388487

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

Table 4 Other materials not included in the PrepSEQ™ Nucleic Acid Extraction Kit

Item	Source
Equipment	
96-Well Magnetic-Ring Stand	AM10050
Block heater, 37°C	MLS
Laboratory mixer (vortex or equivalent)	MLS

Table 4 Other materials not included in the PrepSEQ Nucleic Acid Extraction Kit *(continued)*

Item	Source
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
(Optional, but recommended) Plate centrifuge	MLS
Consumables	
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS
(Optional) MicroAmp™ Clear Adhesive Film	4306311
Reagents	
Ethanol, 95%	MLS
Isopropanol, 100%	MLS
Nuclease-Free Water (not DEPC-Treated)	AM9938

Required materials not included with the kit

Item	Source
Instruments and equipment	
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument	A30304 (desktop) A30299 (laptop) Contact your local microbiology sales representative.
RapidFinder™ Express Software v2.0 or later	Download the latest version at **thermofisher.com/rapidfinder-express-software**
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

Item	Source
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[1]	4358293
MicroAmp™ Optical 8-Cap Strip, 300 strips[1]	4323032
Reagents	
Nuclease-Free Water (not DEPC-Treated)	AM9938

[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.

2 Enrich food samples

Guidelines for sample enrichment

- Use proper aseptic technique while handling samples to avoid cross-contamination.
- Use a forced air incubator and ensure sufficient space between enrichment bags to allow for air flow.

Enrich food samples

1. Prepare Buffered Peptone Water (ISO) (BPW) according to the manufacturer's instructions.
2. Add 225 mL of BPW per 25 g (or 25 mL) of food sample, to the media in the homogenizer bag.
3. Homogenize the sample in a homogenizer bag as described in the following table.

   A filtered bag may be used for enrichment of samples with particulates.

For these food types...	Homogenize by...
Coarse food types, such as meat or poultry[1]	Process for 1–2 minutes in a homogenizer with speed setting **Norm**, or equivalent.
Liquids or powdered foods	Shake the bag at least 25 times to achieve a homogeneous suspension.

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

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

STOPPING POINT *(Optional)* For convenience, enriched cultures can be stored at 5±3°C for up to 72 hours before proceeding to DNA isolation.

3 Isolate DNA with the PrepSEQ™ Nucleic Acid Extraction Kit

Workflow

Before first use of the kit (page 11)

Prepare Binding Solution and Wash Buffer (page 11)

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Set up the processing plates (page 13)

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Process samples on the instrument (page 13)

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, then 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.

Prepare PK Buffer Mix

1. Combine the following components for the number of extractions required plus 10% overage.

Component	Volume per extraction
Proteinase K	10 µL
PK Buffer	140 µL
Total volume per extraction	**150 µL**

2. Mix well to disperse Proteinase K in PK Buffer.

Use PK Buffer Mix immediately or store on ice until ready to use.

Prepare Lysis Buffer Premix

1. Combine the following components for the number of extractions required plus 10% overage.

Component	Volume per extraction
Lysis Buffer	250 µL
Magnetic Particles[1]	30 µL
Binding Solution (isopropanol)	325 µL
Total volume per extraction	**605 µL**

[1] Resuspended and thoroughly mixed.

2. Mix well and store at room temperature (23±5°C).

Store Lysis Buffer Premix at room temperature for up to 2 hours before use. Mix well before dispensing.

Set up the Lysis Plate

1. Collect the enriched sample from the incubator and briefly mix the sample tubes.
2. Set up the Lysis Plate in a Deep Well Plate according to the following table.

Component	Sample well	NEC well[1]
Prepared PK Buffer Mix	150 µL	150 µL
Enriched sample	250 µL	—
Nuclease-free Water	—	250 µL

[1] Reserve at least one well per plate containing Nuclease-free Water as a negative extraction control.

   Pipet up and down 2–3 times to mix after addition of enriched sample or Nuclease-free Water to the PK Buffer Mix.

3. Incubate the Lysis Plate for 10–30 minutes at room temperature.

   During the incubation, set up the instrument processing plates as described in the following section.

Set up the processing plates

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

Plate	Plate type	Action
Tip Comb	Standard	Place a 96-well deep-well tip comb in a standard plate.
Elution Plate	Standard	Add 100 µL of Elution Buffer to each sample and control well.
Wash Plate 1	Deep-Well	Add 300 µL of Wash Buffer to each sample and control well.
Wash Plate 2	Deep-Well	Add 300 µL of Wash Buffer to each sample and control well.

Process samples on the instrument

After incubation of the Lysis Plate is complete:

1. Vortex the prepared Lysis Buffer Premix to ensure even distribution of the Magnetic Particles.
2. Add 605 µL of Lysis Buffer Premix to each well, then pipet up and down 5 times to mix.
3. Select the program on the instrument, then press **Start**.

Instrument	Program
KingFisher™ Flex-96	**4428176PrepSEQ_SM**
MagMAX™ Express-96	**4428176DWPrepSEQFA**

4. Load the prepared plates according to the readout on the instrument, verifying that their orientation is {A1 to A1}.

Plate	Action
Tip Comb	Load the Tip Comb, then press **Start**.
Elution Plate	Load the Elution Plate, then press **Start**.
Wash Plate 1	Load the Wash Plate 1, then press **Start**.
Wash Plate 2	Load the Wash Plate 2, then press **Start**.
Lysis Plate	Load the Lysis Plate, then press **Start**.

5. When processing is complete, after ~45 minutes ("Enjoy your DNA" is displayed on the screen), remove the Elution Plate from the instrument.
   The DNA is in the Elution Plate.

Proceed directly to real-time PCR. Alternatively, seal the plate with MicroAmp™ Clear Adhesive Film and 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 EN ISO 20837:2006.

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

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, then 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 25.

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

If you see this in the Elution Plate...	Do this...
Oil droplets as a top layer	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 1).
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.

(continued)

If you see this in the Elution Plate...	Do this...
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.

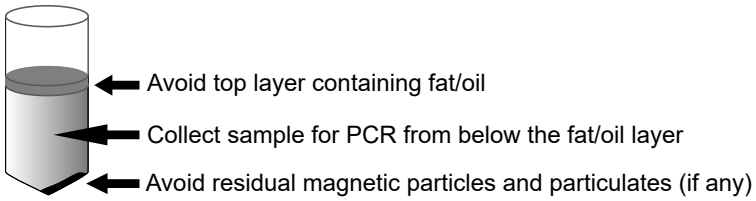

Figure 1 High-fat samples: Collect sample from middle phase after lysis.

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.

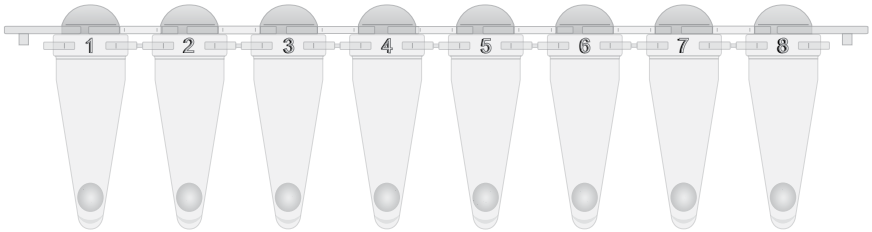

Figure 2 MicroAmp™ Tube Strip labeling The tube strip is shown with tinted dome caps, as shipped. For PCR, replace the dome caps with the optical cap strips provided in the kit.

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**  , then 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

For step-by-step instructions, select **Pipette Samples**  on the main page in RapidFinder™ Express Software.

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 15.

   If the DNA samples have been stored, see “Sample handling” on page 15.

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. Ensure that the reactions are thoroughly mixed: if reactions were not previously mixed during the pipetting step, vortex at high speed for 5–10 seconds.

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

IMPORTANT! If needed, repeat the vortex/centrifugation steps to ensure complete mixing of the samples with the assay beads.

Load and run the reactions

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

1. Transfer the tubes to the 7500 Fast 96W Strip Plate Adaptor in the same configuration as the run layout.

Note: The 7500 Fast 96W Strip Plate Adaptor is not compatible with single Armadillo tubes if the tube frame is broken off or removed.

Be sure to load empty PCR tube strips as directed by the software (Figure 3).

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

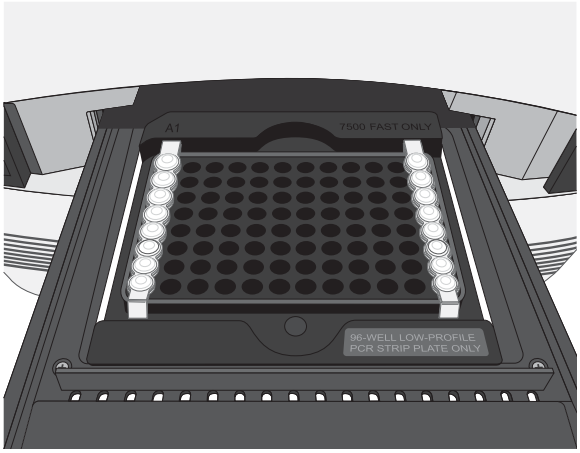

Figure 3 7500 Fast instrument tube layout RapidFinder™ Express Software directs the user to load empty strip tubes in column 1 (far left) and column 12 (far right), if needed. The empty capped 8-tube strips evenly distribute the clamping load applied to the sample tube strips during processing, thereby 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.

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 Confirm positive results

Recommended confirmation methods

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

- Perform a second enrichment step in RVS (0.1 mL BPW in 10 mL RVS broth): incubate at 41.5±1°C for 6–27 hours, then streak onto XLD agar and another selective agar, and incubate at 37±1°C for 24±3 hours. Perform a Latex test (Oxoid™ DR1108) on the observed characteristic colonies.

  Note: In the case of a negative Latex test, perform a biochemical gallery on purified colonies of *Salmonella*. If the confirmatory test remains negative, perform a second selective enrichment in MKTTn broth, and follow the confirmatory tests described in the CEN or ISO standardized methods.

- Using an alternate method certified by NF VALIDATION™ that is based on a different principle than the MicroSEQ™ Salmonella spp. Detection Kit.

  It is necessary that the complete protocol for the second validated method be performed entirely, which means that the enrichment step that precedes the confirmation step must be common to both methods.

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

A Troubleshooting

Observation	Possible cause	Recommended action
A visual difference in PCR beads is observed.	PCR pellets can exhibit differences in morphology.	Ensure thorough pipette mixing followed by vortexing on high speed to confirm pellet is in solution. After PCR, if IPC failure is observed, repeat the reaction.
Eluate volume from DNA sample preparation is less than 90 µL	Evaporation during the elution step.	If three 30-µL PCR reactions are required, add Elution Buffer to the eluate to bring the volume to ~100 µL before proceeding to PCR.
		Remove the Elution Plate from the MagMAX™ Express-96 as soon as processing is complete, to minimize evaporation.
Inhibition of downstream PCR, indicated by nondetection of IPC reaction	Magnetic Particles were in the Elution Plate.	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 *(optional)*: • 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.
	Elution Plate contained incompletely removed particulate residue from the food sample.	Avoid residue during transfer of eluted DNA to the lyophilized assay. *(Optional)* 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.
In positive control wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA existed in the 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 pipette the positive control into all positive control wells.

Observation	Possible cause	Recommended action
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. Additionally, no IPC signal in negative control wells could be caused by: • A high copy number of target DNA existed in the samples, resulting in preferential amplification of the target-specific DNA. • A problem occurred with IPC amplification.	To correct carryover contamination, repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.
		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 extraction control wells, target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred.	1. Repeat the assay using fresh aliquots of all reagents, fresh enrichment, and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
In 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, then 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.
In unknown wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA existed in the 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 occurred.	After adding 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, then centrifuge the strips at 200–600 × *g* for about 10 seconds. 2. Vortex the strips again on high speed for about 10 seconds, then 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 did 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, then 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.

B

Supplemental information

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*.

NF VALIDATION™ by AFNOR™ Certification

Table 5 NF VALIDATION™ by AFNOR™ Certification of the workflow

Certification	Expiration
BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140 ABI 29/02 - 09/10 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS http://nf-validation.afnor.org/en	For information about the end of validity of the NF VALIDATION™ by AFNOR™ Certification, see the certificate, ABI 29/02 – 09/10, available at **nf-validation.afnor.org/en** or **thermofisher.com/foodsafety**.

The MicroSEQ™ Salmonella spp. Detection Kit has been certified by AFNOR™ Certification. The certification uses the EN ISO 16140 standard for the validation of alternative methods (Alternative Analytical Methods for Agribusiness. Certified NF VALIDATION™ by AFNOR™ Certification; **nf-validation.afnor.org/en**). This kit was compared and found equivalent to the EN ISO 6579 reference method. The validated workflow described in this user guide includes:

- Enrichment in BPW
- The PrepSEQ™ Nucleic Acid Extraction Kit
- The MicroSEQ™ Salmonella spp. Detection Kit
- The Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software v2.0 or later

- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 6 for characteristics) with RapidFinder™ Express Software v2.0 or later.

Table 6 7500 Fast Real-Time PCR Instrument characteristics

Characteristics	7500 Fast Real-Time PCR Instrument
Optics	12v 75w halogen bulb
Filters	5 excitation and 5 emission filters
Sample ramp rate	Standard mode: ±1.6°C/sec Fast mode: ±3.5°C/sec
Thermal range	4-100°C
Thermal accuracy	±0.5°C
Thermal uniformity	±1°C
Format	96-well, 0.1-mL block

- Confirmation testing as described in "Recommended confirmation methods"

Table 7 Validated matrices

Reference method	Matrix
EN ISO 6579 (2017): Horizontal method for the detection of *Salmonella* spp.	Meat products (processed and unprocessed): Poultry, pork, and beef

General remarks and recommendations:

- Comply with Good Laboratory Practices (GLP; refer to EN ISO 7218:2024 standard).
- Follow EN ISO 6579 and EN ISO 6887 standards for the preparation of initial suspensions.
- In the context of NF VALIDATION™ by AFNOR™ Certification, samples of more than 25 grams have not been tested.

Good laboratory practices for PCR

Note: Spin tubes/plates before performing PCR. Spinning of PCR tubes is most easily accomplished by using a centrifuge designed for PCR tubes or plates. Follow manufacturer instructions for loading tubes/plates.

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

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.

- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Do not open reaction tubes after PCR.
- Do not autoclave reaction tubes after PCR.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA*Zap*™ Solutions (Cat. No. AM9890) according to the Thermo Fisher Scientific PCR Decontamination Protocol. After cleaning with bleach we recommend a rinse with distilled water or an ethanol solution because bleach will rust stainless steel. Note that minor discoloration of metal parts may occur.

For additional information, refer to EN ISO 22174:2024 or **www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html**.

C

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

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

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

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at:
  cdc.gov/labs/bmbl
- World Health Organization, *Laboratory Biosafety Manual*, 4th Edition, WHO/CDS/CSR/LYO/2020.12; found at:
  who.int/publications/i/item/9789240011311

D Documentation and support

Food safety support

Website: **https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html** or **thermofisher.com/foodsafety**

Support email:

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

Phone: Visit **thermofisher.com/support**, select the link for phone support, then select the appropriate country from the dropdown list.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Related documentation

Document	Publication number
RapidFinder™ Express Software Quick Reference	4480999
Thermo Scientific™ KingFisher™ Flex User Manual	N07669

(continued)

Document	Publication number
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4378657
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide	4387777
PCR Starter Kit for 96-well blocks, 0.2 mL, User Guide	A24829

References

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

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

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

EN ISO 7218:2024. Microbiology of the food chain – General requirements and guidance for microbiological examinations.

EN ISO 16140:2-2016. Microbiology of the food chain – Method Validation - Part 2:Protocol for the validation of alternative (proprietary) methods against a reference method.

EN ISO 20837:2006. Microbiology of the food and animal feeding stuffs – PCR for the detection of food-borne pathogens. Requirements for sample preparation for qualitative detection.

EN ISO 22174:2024. Microbiology of the food chain – PCR for the detection of food-borne pathogens. General requirements and definitions.