applied biosystems

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

USER GUIDE

Using automated or spin-column-based DNA isolation methods

for use with:
PrepSEQ™ Nucleic Acid Extraction Kit
PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K
MicroSEQ™ Salmonella spp. Detection Kit
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
RapidFinder™ Express Software v2.0 or later

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FOR AGRIBUSINESS
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For testing of Food and Environmental samples only.

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Revision history: Pub. No. 4476867 (English)

Revision	Date	Description
E	04 August 2020	• Updated software version for RapidFinder™ Express Software. • Added characteristics of the 7500 Fast Real-Time PCR Instrument.
D	07 August 2019	Moved the legal statement from the front cover to page 2.
C	16 January 2019	• Changed storage temperature of Magnetic Particles from 5±3°C to ambient temperature (15°C to 30°C). • Aligned storage temperatures of other kit components to the temperatures displayed on the product labels. • Changed instructions so that assay beads are equilibrated at room temperature and DNA samples are added to beads at room temperature (23±5°C). • Moved enrichment and confirmation instructions to individual chapters, for alignment to current style and template. • Reorganized and moved instructions for PCR setup and analysis with SDS software to an appendix. • Removed instructions for the StepOne™ and StepOnePlus™ instruments (not part of the validated workflows).
B	September 2014	• Updated NF VALIDATION™ certification wording to most recent requirements. • Updated template with associated updates to the limited use label license information, warranty information, trademark statement, and safety statements.
A	November 2012	New user guide.

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.

Product description

We have developed a sample preparation workflow for detection of *Salmonella* spp. in primary production samples. It is approved by the National Poultry Improvement Plan. The workflow outlines enrichment, sample preparation, and detection by real-time PCR. The following sample preparation methods are available for use in detection of *Salmonella* spp. in primary production samples post-enrichment:

- Magnetic bead-based method — see Chapter 3, "Isolate DNA with the PrepSEQ™ Nucleic Acid Extraction Kit"
- Spin column-based method — see Chapter 4, "Isolate DNA with PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K"

Both methods use a shared two-step enrichment before sample purification.

Required materials

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

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

Materials for enrichment

Item	Source
Equipment	
Incubator, 37±1°C	MLS
Homogenizer Laboratory Blender	DB5000A, or equivalent
Diluflux™ Pro Automated Gravimetric Dilutor	DB4150A, DB4100A, or equivalent
Homogenizer bag appropriate for the sample type and size	
Homogenizer bag BagFilter™ 400	DB4011A, or equivalent
Homogenizer bag BagPage™ 400	DB4012A, or equivalent
Homogenizer bag BagLight™ 400	DB4013A, or equivalent
Homogenizer bag RollBag™ 1300	DB4014A, or equivalent
Reagents	
Tetrathionate Broth (TT Broth Base), I_2KI, and Brilliant Green	Remel™ R454832, or equivalent
Buffered Peptone Water (ISO)	DF1049, CM1211B, or equivalent

Materials for DNA Isolation using the PrepSEQ™ NAE kit

Table 1 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	
Elution Buffer	25 mL	3 × 25 mL	

Table 1 PrepSEQ Nucleic Acid Extraction Kit *(continued)*

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

[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 2 Magnetic particle processor

Item	Source
KingFisher™ Flex-96 instrument and accessories	
KingFisher™ Flex-96 Deep Well Magnetic Particle Processor	A32681, 96 DW plate, or equivalent[1]
KingFisher™ Deepwell 96 Plate, V-bottom	95040450
KingFisher™ 96 KF microplates (200 µL)	97002540
KingFisher™ Flex 96 KF Heating Block	24075420
KingFisher™ 96 tip comb for DW 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 DW magnetic head is required (Cat. No. 24074430).

Table 3 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 3 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	AM9938

Materials for DNA isolation using the PrepSEQ™ Rapid Spin kit

Table 4 PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K (100 reactions, Cat. No. 4426715)

Contents	Amount	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
Proteinase K (20 mg/mL), 1 tube	1.25 mL	Below –18°C

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

Table 5 Required materials not included in the PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K

Item	Source
Equipment	
Block heaters, 56°C and 97°C	MLS
Rack for 1.5-mL tubes	MLS
Benchtop microcentrifuge	Eppendorf 5415 D or equivalent

Table 5 Required materials not included in the PrepSEQ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K *(continued)*

Item	Source
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	AM9938

Materials for PCR detection

Table 6 MicroSEQ™ Salmonella spp. Detection Kit [96 reactions; Cat. Nos. 4403930, 4412639 (includes the PrepSEQ™ Nucleic Acid Extraction Kit)]

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

[1] See the product label for the expiration date.

[2] 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.

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

Table 7 Required materials not included with the MicroSEQ™ kit

Item	Source
Instruments and equipment	
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument	A30304 (desktop) A30299 (laptop) Contact your local microbiology sales representative.

Item	Source
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
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	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.

Workflow

Enrich environmental samples (page 13)

▼

Isolate DNA with the PrepSEQ™ Nucleic Acid Extraction Kit (page 15)

▼

or

Isolate DNA with PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K (page 19)

▼

PCR with the MicroSEQ™ Salmonella spp. Detection Kit and RapidFinder™ Express Software (page 22)

▼

Confirm positive results (page 29)

2 Enrich environmental 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 production environmental samples—primary enrichment

1. Prepare the TT Broth according to the manufacturer's recommendation.
2. Add media and samples in a homogenizer bag, as indicated.

Sample type	Volume of media/sample	Media
Boot socks	100–150 mL	Tetrathionate Broth (TT Broth Base), I_2KI (20 mL/L), and Brilliant Green (10 mL/L)
Sponges	100–150 mL	
Swabs	10 mL	
• Solid samples (feces) • Liquid samples	225 mL per 25-g or 25-mL sample [1]	

[1] 1-in-10 ratio

3. Homogenize the sample for approximately 1 minute under normal speed.
4. Incubate the sample at 37±1°C for 16–20 hours under static conditions.

Enrich production environmental samples—secondary enrichment

1. In an appropriate container, dilute the enriched sample with Buffered Peptone Water (BPW) at a 1-in-9 ratio of sample to media.

   For example, combine 1 mL of sample with 9 mL of BPW in an appropriate container.
2. Incubate the sample at 37±1°C for 5±1 hours under static conditions.

3. Briefly mix the sample to ensure that bacteria are in solution; for example, vortex for about 5 seconds.

4. Proceed to DNA isolation (Chapter 3, Isolate DNA with the PrepSEQ™ Nucleic Acid Extraction Kit or Chapter 4, Isolate DNA with PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K).

   Note: Retain sufficient BPW sample for confirmation or repeat testing of presumptive positives. Store samples at 5±3°C.

3 Isolate DNA with the PrepSEQ™ Nucleic Acid Extraction Kit

Workflow

Resuspend Magnetic Particles (page 16)

▼

Prepare Binding Premix Solution (page 16)

(450 µL PK Buffer + 25 µL Magnetic Particles + 325 µL Binding Solution)

▼

Set up the processing plates (page 17)

(100 µL enriched culture)

▼

Process samples on the instrument (page 17)

(KingFisher™ Flex-96 instrument: 4428176PrepSEQ_SPP)

(MagMAX™ Express-96 instrument: 4428176DWPrepSEQFA)

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.

Prepare Binding Premix Solution

1. Combine the following components of the number of samples required.

Component	Volume per sample	Volume for *n* samples[1]
PK Buffer	450 µL	495 µL × *n*
Magnetic Particles[2]	25 µL	27.5 µL × *n*
Binding Solution (isopropanol)	325 µL	357.5 µL × *n*
Total volume per extraction	800 µL	880 µL × *n*

[1] Includes 10% overage.
[2] Resuspended and thoroughly mixed.

2. Mix well by vortexing for approximately 5 seconds until resuspension is complete.

   Use the Binding Premix Solution within 1 hour of preparation. Store at room temperature.
3. Add 800 µL of Binding Premix Solution to each well of the Lysis (sample) Plate.
4. When instructed by the instrument, load the plate into the instrument.

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 120 µL of Elution Buffer to each sample and control well. **Note:** To include the Elution Buffer Control, add 120 µL of Elution Buffer to an extra empty well in the Elution Plate.
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.
Lysis (sample) Plate	Deep Well	Add 100 µL pre-enriched BPW liquid sample, prepared in "Enrich production environmental samples—secondary enrichment" on page 13. Add from the filtered side of the enriched sample culture bag(s) to the Lysis (sample) Plate containing 800 µL of Binding Premix Solution.

Process samples on the instrument

1. Select the program on the instrument, then press **Start**.

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

2. 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 (sample) Plate	Load the Lysis (sample) Plate, then press **Start**.

3. When processing is complete, the message "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.

4 Isolate DNA with PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K

Workflow

Filter 750 µL of enriched culture through the spin column

(750 µL enriched culture)

▼

Lyse the sample (page 21)

(55 µL Proteinase K-Lysis Buffer)

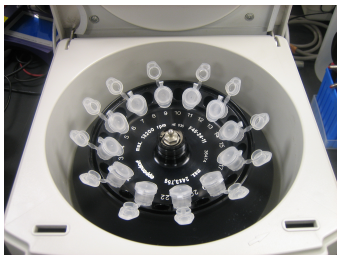
Guidelines for DNA isolation

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.

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.

Before each use of the kit

- Prepare Proteinase K-Lysis Buffer: combine the following components for the number of samples required; store on ice until use.

Component	Volume per sample	Volume for *n* samples[1]
Proteinase K, 20 mg/mL	5 µL	5.5 µL × *n*
Lysis Buffer	50 µL	55 µL × *n*

[1] Includes 10% overage.

- Preheat block heaters to 97±2°C and 56±1°C.

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 19.

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 20.

Lyse the sample

1. Add 55 µL of Proteinase K-Lysis Buffer to the pellet, and pipet up and down or vortex until the pellet is well dispersed.

2. 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 56±1°C for at least 30 minutes.

4. Incubate at 97±2°C for 12±2 minutes, then 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.

5 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

- For the PrepSEQ™ Nucleic Acid Extraction Kit —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).
- For the PrepSEQ™ Rapid Spin Sample Preparation Kit —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.
- For the Pathogen Detection Negative Control — Vortex then briefly centrifuge the tubes.
- 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 35.

PrepSEQ™ Nucleic Acid Extraction Kit — Avoid the fat layer and particles after sample lysis

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).
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.

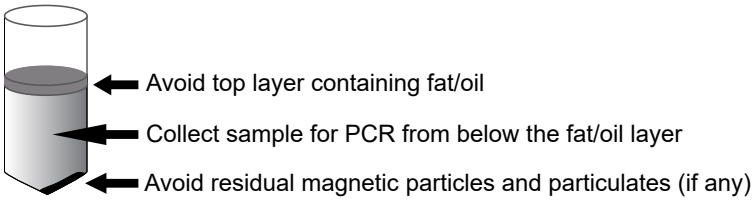


Figure 1 High-fat samples: Collect sample from middle phase after lysis.

PrepSEQ™ Rapid Spin Sample Preparation Kit — Avoid the fat layer after sample lysis

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.

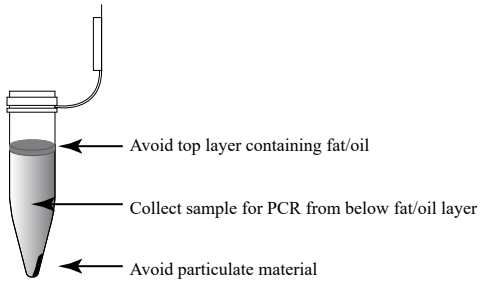


Figure 2 High-fat samples: collect sample from middle phase after lysis.

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.

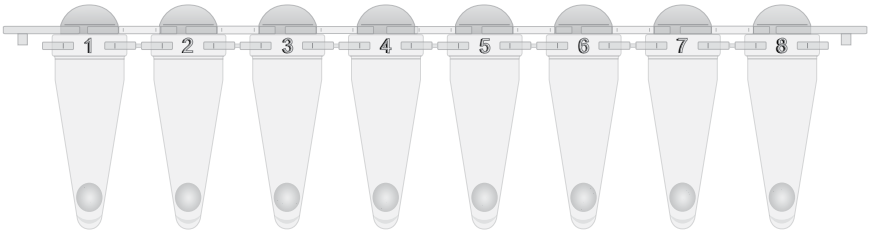


Figure 3 MicroAmp™ Tube Strip labeling

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, see “Sample handling” on page 22.
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. Use the PCR carry plate to 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 low profile PCR tubes as directed by the software (Figure 4).

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

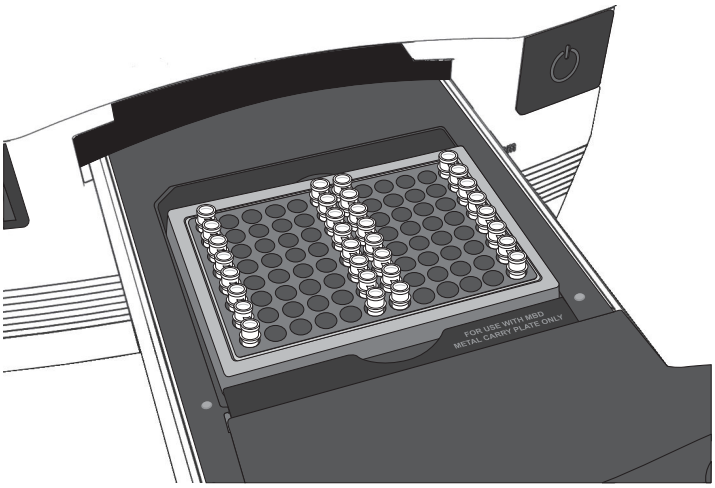


Figure 4 7500 Fast instrument tube layout

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.

6 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.

- Using conventional tests described in the methods standardized by CEN or ISO from colonies (including the purification step). The confirmation step must start from the primary enrichment broth.
- Perform a second enrichment step in Rappaport-Vassiliadis Soya Peptone (RVS) broth (0.1 mL BPW in 10 mL of RVS broth): incubate at 41.5±1°C for 24±3 hours, and streak onto Xylose Lysine Deoxycholate (XLD) agar or another selective agar plate. Then either perform a Latex test (Oxoid™ FT0203A) on the observed characteristic colonies, or perform a biochemical gallery on purified colonies characteristic of *Salmonella.*
- Any other method certified by NF VALIDATION™ that is based on a different principle than the MicroSEQ™ *Salmonella* spp. method. It is necessary that the complete protocol of the second validated method be performed entirely, which means that all steps that precede 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.

Troubleshooting

Troubleshooting automated DNA extraction

Observation	Possible cause	Recommended action
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 contains 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.
	Removal of sample supernatant before addition of lysis buffer was incomplete.	Ensure maximal removal of the supernatant without disturbing the bacterial pellet.

Troubleshooting spin-column-based DNA extraction

Observation	Possible cause	Recommended action
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 .
	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.
The bacterial pellet is difficult to avoid during aspiration.	The sample was left unattended before aspirating off the supernatant, causing dissipation of the bacterial pellet.	Re-centrifuge and remove the supernatant immediately following centrifugation.
The bacterial pellet is difficult to see.	The size of the bacterial pellet is very small.	Remove the supernatant carefully, leaving up to 50 µL of supernatant in the tube, to avoid aspiration of pellet.

Troubleshooting PCR

Observation	Possible cause	Recommended action
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.
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 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.

Observation	Possible cause	Recommended action
In negative extraction control wells, target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred.	1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
In negative 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.
	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.

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

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

Table 8 NF VALIDATION™ by AFNOR™ Certification of the workflow

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

The PrepSEQ™ Nucleic Acid Extraction Kit, the PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K, and MicroSEQ™ *Salmonella* spp. Detection Kit are part of the NF VALIDATION™ workflow to recover *Salmonella* spp. DNA from broth cultures of environmental samples commonly found in the primary production stage. The certification uses the EN ISO 16140 standard for the validation of alternative methods (Alternative Analytical Methods for Agribusiness. Certified by NF VALIDATION™; **nf-validation.afnor.org/en**). This kit was compared and found

equivalent to the EN ISO 6579 reference method. It is approved by the National Poultry Improvement Plan. The validated workflow includes:

- One of the following sample preparation kits:
  - PrepSEQ™ Nucleic Acid Extraction Kit
  - PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K
- MicroSEQ™ Salmonella spp. Detection Kit
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 9 for characteristics) with RapidFinder™ Express Software v2.0 or later.

Table 9 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℃/sec Fast mode: ±3.5℃/sec
Thermal range	4-100℃
Thermal accuracy	±0.5℃
Thermal uniformity	±1℃
Format	96-well, 0.1-mL block

Table 10 Validated matrices

Reference method	Matrix
EN ISO 6579:2017	Environmental samples: boot socks (poultry and pigs), feces (poultry and pigs), litter, animal drinking water, and sponges
NF U47-100; 2007	

General remarks and recommendations:

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

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 DNA*Zap*™ Solutions (Cat. No. AM9890).

For additional information, refer to EN ISO 22174:2005 or **www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html**.

Safety

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.

- 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:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.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**

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

Document	Publication number
RapidFinder™ Express Software Quick Reference	4480999
Thermo Scientific™ KingFisher™ Flex User Manual	N07669
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4378657

(continued)

Document	Publication number
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 6887-2:2017. 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 7218:2007/Amd 1: 2013. Microbiology of food and animal feeding stuffs— 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 22174:2005. Microbiology of the food and animal feeding stuffs– PCR for the detection of food-borne pathogens. General requirements and definitions.

2012. NF Validation by AFNOR-Certification. **nf-validation.afnor.org/en**. Accessed October 2015.

NF U47-100. French norm on isolation and identification of Salmonella spp. in the environment and samples from animal productions: “Recherche par l’isolement et l’identification de tout sérovar ou de sérovar(s) spécifié(s) de salmonelles dans l’environnement des productions animales (Detection by streaking of any Salmonella serotypes in primary production samples).