

Real-Time PCR Detection of *Listeria* spp. in Food and Environmental Samples

USER GUIDE

Using spin-column-based DNA isolation methods

for use with:

PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K

PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K

MicroSEQ™ *Listeria* spp. Detection Kit

Applied Biosystems™ 7500 Fast Real-Time PCR Instrument

RapidFinder™ Express Software v2.0 or later

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For testing of Food and Environmental samples only.

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Revision history: MAN0014554 B (English)

Revision	Date	Description
B	27 August 2024	<ul style="list-style-type: none">• Troubleshooting was added for possible instance of varying morphology of PCR pellets.• Updated software version for RapidFinder™ Express Software.• Added characteristics of the 7500 Fast Real-Time PCR Instrument.
A.0	2 November 2018	<p>New document for the <i>Real-Time PCR Detection of Listeria spp. in Food and Environmental Samples User Guide (Spin-Column DNA Isolation, AOAC)</i>.</p> <ul style="list-style-type: none">• Includes the complete AOAC <i>Performance Tested Methods</i>™ workflow that covers enrichment, DNA isolation, and real-time PCR detection.• Supersedes:<ul style="list-style-type: none">– <i>PrepSEQ™ Rapid Spin Sample Preparation Kit: Listeria spp.</i> (Pub. No. 4426506)– <i>MicroSEQ™ Listeria Detection Kits User Guide</i> (Pub. No. 4489329)

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

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Contents

■	CHAPTER 1 Overview	5
	General overview	5
	Required materials	5
	Materials for enrichment of food samples	6
	Materials for DNA isolation	7
	Materials for PCR detection	8
■	CHAPTER 2 Enrich food or environmental samples	10
	Guidelines for sample enrichment	10
	Enrich food samples	10
	Enrich environmental swab samples	11
	Enrich environmental sponge samples	11
■	CHAPTER 3 Isolate DNA with PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K	12
	Workflow	12
	Guidelines for DNA isolation	12
	PCR-clean water	12
	Position of the spin column/tube assembly in the microcentrifuge	12
	For high-fat samples: remove fat layer before lysis	13
	Before each use of the kit	13
	Filter 750 µL of enriched culture through the spin column	13
	Lyse the sample	14
■	CHAPTER 4 Perform PCR with the MicroSEQ™ Listeria spp. Detection Kit and RapidFinder™ Express Software	15
	Important procedural guidelines for PCR	15
	Software	15
	Sample handling	15
	MicroAmp™ tube strips	16
	Create or edit a run file in RapidFinder™ Express Software	16
	Prepare the assay beads	17
	Set up the PCR reactions	17

Load and run the reactions	18
View results and data analysis	18
■ CHAPTER 5 Recommended confirmation methods	19
■ APPENDIX A Troubleshooting	20
■ APPENDIX B Supplemental information	23
Kit specificity	23
Kit sensitivity	23
AOAC <i>Performance Tested Methods</i> SM Certification	23
Good laboratory practices for PCR	24
■ APPENDIX C Safety	26
Chemical safety	26
Biological hazard safety	27
■ APPENDIX D Documentation and support	28
Food safety support	28
Customer and technical support	28
Related documentation	28
References	30



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

General overview

This guide describes the following AOAC Research Institute *Performance Tested Methods*SM-certified workflow for detection of *Listeria* spp. in food and environmental surfaces:

1. Enrichment of 25 g or 25 mL food samples or of environmental samples in Buffered Listeria Enrichment Broth (BLEB).
2. Spin-column-based preparation of PCR-ready DNA using a PrepSEQ™ Rapid Spin Sample Preparation Kit.

Sample type	Recommended kit
<ul style="list-style-type: none">• Food without high fat content• Environmental samples	PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K
Food with high fat content	PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K

3. Real-time PCR detection of *Listeria* spp. DNA using the MicroSEQ™ *Listeria* spp. Detection Kit and RapidFinder™ Express Software on the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument.
4. Confirmation testing of positive samples.

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

Go to [thermofisher.com/foodsafety](https://www.thermofisher.com/foodsafety) for a list of workflows for detection of *Listeria* (Pub. No. MAN0009418).

Required materials

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or [thermofisher.com](https://www.thermofisher.com). They may also be available through Fisher Scientific ([fisherscientific.com](https://www.fisherscientific.com)), MLS, or another 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

Item	Source
Equipment	
Incubator, 30±1°C	thermofisher.com
Homogenizer laboratory blender or diluter, one of the following or equivalent:	
<ul style="list-style-type: none"> 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 or equivalent DB4150A or equivalent
Consumables	
For environmental samples only:	
Swab, cotton	MLS
15-mL conical tubes	MLS
Homogenizer bag appropriate for the sample type	
For the Homogenizer Laboratory Blender or equivalent:	
Homogenizer bag, with sponge, 4.5" × 9" (Whirl-Pak™ Speci-Sponge™ Environmental Sampling Bag, or equivalent)	Nasco B01245WA or equivalent
Homogenizer bag, with mesh, 6" × 9", 24 oz (Whirl-Pak™ Filter Bag for Homogenizer Blenders, or equivalent)	Nasco B01348WA or equivalent
Homogenizer bag, 6" × 9", 24 oz (Whirl-Pak™ Sample Bag, or equivalent)	Nasco B01297WA or equivalent
For the Diluflux™ Automated Gravimetric Dilutor :	
Homogenizer bag BagFilter™ 400	DB4011A or equivalent
Homogenizer bag BagPage™ 400	DB4012A or equivalent
Homogenizer bag BagLight™ 400	DB4013A or equivalent
Reagents	
Dey Engley (D/E) Neutralizing Broth (for swab or sponge samples collection)	R453042 or equivalent
Oxoid™ Buffered Listeria Enrichment Broth (BLEB), 500 g	CM0897B
Oxoid™ Listeria Selective Enrichment Supplement	SR0141E

Materials for DNA isolation

Choose the PrepSEQ™ Rapid Spin Sample Preparation Kit appropriate for your sample type.

Table 1 PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K (100 reactions, Cat. No. [4426714](#))

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

^[1] See the expiration date on the box.

Table 2 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] See the expiration date on the box.

Table 3 Required materials not included in the PrepSEQ™ Rapid Spin Sample Preparation Kit

Item	Source
Equipment	
Block heaters, 56°C and 95°C	MLS
Rack for 1.5-mL tubes	MLS
Benchtop microcentrifuge	Eppendorf 5415 D or equivalent
Laboratory mixer, Vortex or equivalent	MLS
Pipettors: <ul style="list-style-type: none"> • Positive-displacement • Air-displacement 	MLS
Additional consumables	
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS

Table 3 Required materials not included in the PrepSEQ Rapid Spin Sample Preparation Kit (continued)

Item	Source
Reagents	
Nuclease-Free Water (not DEPC-Treated)	AM9938

Materials for PCR detection

Table 4 MicroSEQ™ *Listeria* spp. Detection Kit (96 reactions; Cat. No. [4427410](#))

Contents	Amount	Storage
<i>Listeria</i> spp. Assay Beads, 8-tube strips in rack (pink rack)	12 strips (96 tubes)	5±3°C Protect from light and moisture. ^[1]
MicroAmp™ Optical 8-Cap Strips	12 strips (96 caps)	
Pathogen Detection Negative Control (red cap) ^[2]	1.5 mL	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.

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
Laboratory mixer (vortex mixer or equivalent)	MLS

Item	Source
Pipettors: <ul style="list-style-type: none"> • 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.



Enrich food or 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 food samples

1. Prepare Buffered Listeria Enrichment Broth (BLEB) according to the manufacturer's instructions.
2. Add 225 mL of BLEB to 25 g (or 25 mL) of food sample.
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 or soft food types ^[1]	Process for 1 minute in a laboratory blender.
<ul style="list-style-type: none">• Liquids or powdered foods^[1]• Solid foods^[1]	Hand squeeze the bag 5–10 times.

^[1] See "AOAC Performance Tested MethodsSM Certification" on page 23 for validated matrices.

4. Incubate the sample for 4 ± 0.25 hours at $37 \pm 1^\circ\text{C}$ under static conditions, then add Listeria Selective Enrichment Supplement, as directed by the manufacturer.
Follow the initial incubation time specified by the manufacturer.
5. Continue the incubation for a total of 22–24 hours at $37 \pm 1^\circ\text{C}$ under static conditions.

Enrich environmental swab samples

1. Prepare Buffered Listeria Enrichment Broth (BLEB) according to the manufacturer's instructions.
2. Prewet the swab with 0.5 mL of D/E Neutralizing Broth, if necessary, then wipe the surface area to be tested.
3. Add the swab to 10 mL of BLEB in a 15-mL conical tube.
4. Twirl the swab for 90 ± 30 seconds.
5. Incubate the sample for 4 ± 0.25 hours at $37\pm 1^\circ\text{C}$ under static conditions, then add Listeria Selective Enrichment Supplement, as directed by the manufacturer.
Follow the initial incubation time specified by the manufacturer.
6. Continue the incubation for a total of 28–32 hours at $37\pm 1^\circ\text{C}$ under static conditions.

Enrich environmental sponge samples

1. Prepare Buffered Listeria Enrichment Broth (BLEB) according to the manufacturer's instructions.
2. Pre-wet the sponge with 10 mL of D/E Neutralizing Broth, if necessary, then wipe the surface area to be tested.
3. Add the sponge to 100 mL of BLEB in a homogenizer bag without mesh.
4. Hand squeeze the bag 5–10 times.
5. Incubate the sample for 4 ± 0.25 hours at $37\pm 1^\circ\text{C}$ under static conditions, then add Listeria Selective Enrichment Supplement, as directed by the manufacturer.
Follow the initial incubation time specified by the manufacturer.
6. Continue the incubation for a total of 28–32 hours at $37\pm 1^\circ\text{C}$ under static conditions.

3

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

(55 µL Proteinase K-Lysis Buffer)

(Optional transfer to clean tube, for high-fat samples)

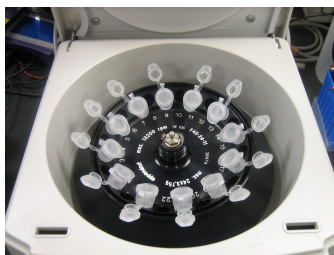
Guidelines for DNA isolation

PCR-clean water

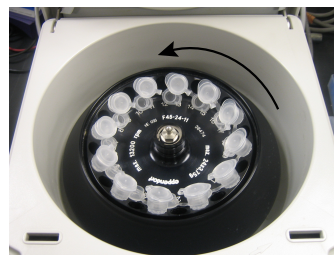
Use nuclease-free water for all procedures described in this protocol that require water. Nuclease-free water is considered “PCR-clean” water. In contrast, autoclaved water should not be considered "PCR-clean" water.

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	<ol style="list-style-type: none"> 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	<ol style="list-style-type: none"> 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 μ L	5.5 μ L $\times n$
Lysis Buffer	50 μ L	55 μ L $\times n$

^[1] Includes 10% overage.

- Preheat block heaters to $97\pm 2^\circ\text{C}$ and $56\pm 1^\circ\text{C}$.

Filter 750 μ L 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 μ L 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 $\times g$ 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 13.

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. *(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 $56\pm 1^\circ\text{C}$ for at least 30 minutes.
4. Incubate at $97\pm 2^\circ\text{C}$ for 12 ± 2 minutes, then allow the sample to cool for about 2 minutes at room temperature ($23\pm 5^\circ\text{C}$).
5. Microcentrifuge the tube at $12,000\text{--}16,000 \times g$ 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 $12,000\text{--}16,000 \times g$ 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 $5\pm 3^\circ\text{C}$ 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™ Listeria 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 have been stored or the pellet has dispersed, thaw the samples (if necessary), vortex, then 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 24.

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.

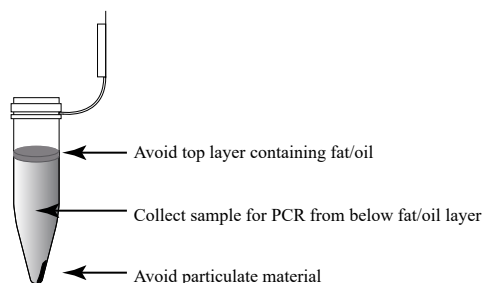


Figure 1 High-fat samples: collect sample from middle phase after lysis

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 $5\pm 3^{\circ}\text{C}$.
- 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.

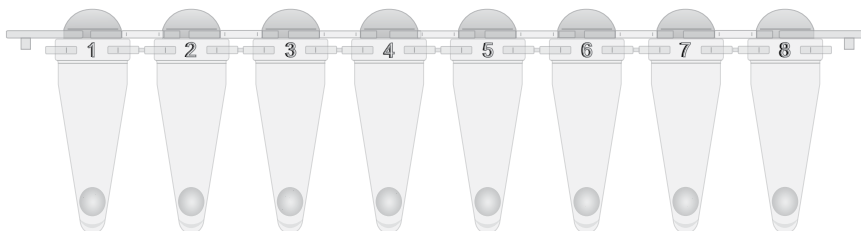



Figure 2 MicroAmp™ Tube Strip labeling The tube strip is shown with tinted dome caps, as shipped. For PCR, replace the dome caps with the optical cap strips provided in the kit.

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 ($23\pm 5^{\circ}\text{C}$).
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, then mix each sample or control thoroughly. If the DNA samples have been stored or the pellet has dispersed, see “Sample handling” on page 15.
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 15.
2. Following the layout determined by RapidFinder™ Express Software, add 30 μL of sample or control to each assay bead at room temperature ($23\pm 5^{\circ}\text{C}$), then 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 $200\text{--}600 \times g$ for about 20 seconds using a centrifuge with a plate adapter or a benchtop microcentrifuge with an 8-strip PCR tube adapter.

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, 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 3).

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

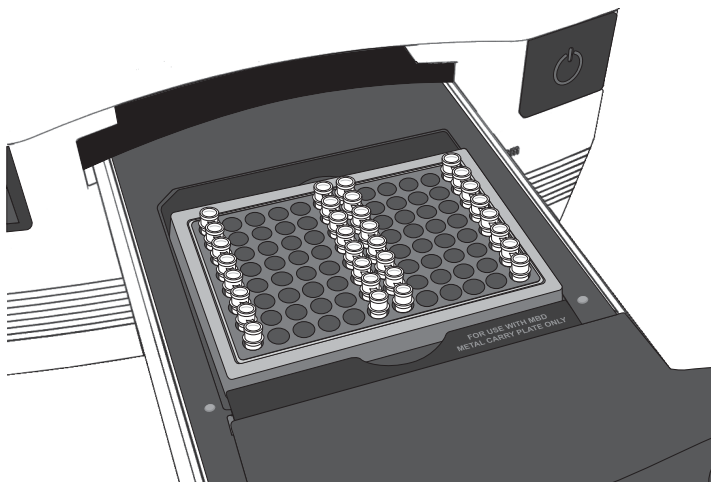



Figure 3 7500 Fast instrument tube layout RapidFinder™ Express Software directs the user to load empty strip tubes in column 1 (far left) and column 12 (far right), if needed. The empty capped 8-tube strips evenly distribute the clamping load applied to the sample tube strips during processing, thereby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

View results and data analysis

Data analysis is automated by the software.

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

To display a list of results in table format, click **Table View**. Select a sample, then click **View Details** to see replicate information about samples.



Recommended confirmation methods

In the context of AOAC Research Institute *Performance Tested Methods*SM certification, enriched cultures with positive PCR results were tested further by cultural confirmation following ISO 11290-1.



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.
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 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 was 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 was associated with PCR-inhibitory components.	Pre-wash the bacterial pellet before loading the Rapid Spin column: <ol style="list-style-type: none"> 1. Transfer 750 µL of sample to a clean microcentrifuge tube. 2. Centrifuge at 12,000–16,000 × <i>g</i> for about 3 minutes. 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 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:	To correct carryover contamination, repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.
	<ul style="list-style-type: none"> 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 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.	<ol style="list-style-type: none"> Repeat the assay using fresh aliquots of all reagents, fresh enrichment, and clean pipetting equipment. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 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.	<p>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.</p> <p>Refer to other troubleshooting suggestions for removal of particulates from the DNA sample.</p>
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.	<p>After adding 30 µL of sample or Pathogen Negative Control to the bead and capping the tubes:</p> <ol style="list-style-type: none"> Vortex strips at high speed for about 10 seconds, then centrifuge the strips at 200–600 × g for about 10 seconds. Vortex the strips again on high speed for about 10 seconds, then centrifuge the strips at 200–600 × g for about 1 minute. <p>Ensure that all liquid is at the bottom of the tubes and the beads are fully dissolved before proceeding.</p>

Observation	Possible cause	Recommended action
Replicate results for a sample are inconsistent.	All replicate wells for a sample did not have the same result.	<p>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.</p> <p>If only 2 replicates were run and the results are not consistent, repeat the assay using fresh samples and reagents.</p>
Amplicon contamination.	<ul style="list-style-type: none"> • 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. 	<p>To confirm amplicon contamination, perform the following experiment:</p> <p>Prepare negative control samples using at least one 8-tube strip of MicroSEQ™ Assay Beads.</p> <ol style="list-style-type: none"> 1. Divide the assay beads into two sets. <ol style="list-style-type: none"> 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: <ul style="list-style-type: none"> • 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. <p>Amplicon contamination is indicated by target-specific signal in the –UNG samples and no target-specific signal in +UNG samples.</p> <p>If the instrument block was contaminated, consult the <i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve</i> (Pub. No. 4347825) and/or contact a service representative to clean the instrument.</p>



Supplemental information

Kit specificity

The MicroSEQ™ *Listeria* spp. Detection Kit can detect the following *Listeria* species: *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri*, *L. grayi*, and *L. marthi*.

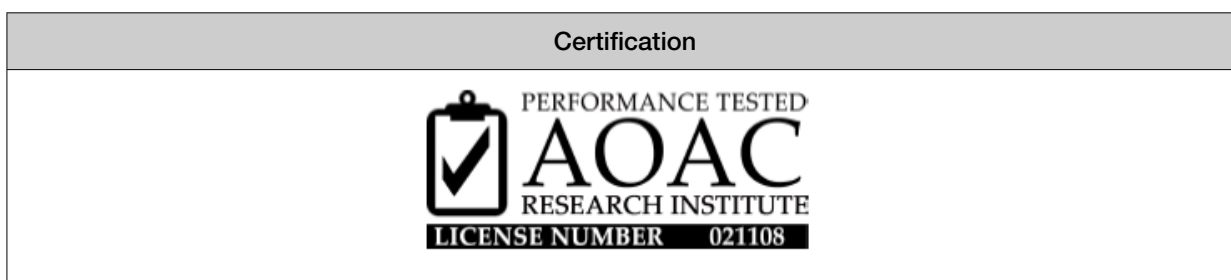
Kit sensitivity

The sensitivity of the assay in culture samples depends on the quality of the sample preparation method that is used. The AOAC-RI *Performance Tested Methods*™ workflow described in this user guide allows you to detect 1 to colony-forming units (CFU) from 25 grams or 25 mL of food.

Go to thermofisher.com/foodsafety for a list of workflows for detection of *Listeria* (Pub. No. MAN0009418).

AOAC Performance Tested Methods™ Certification

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



The detection of *Listeria* spp. using PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K and PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K and the MicroSEQ™ *Listeria* spp. Detection Kit has earned the AOAC *Performance Tested Methods*™ Certification from the AOAC Research Institute. The certified workflow described in this user guide includes:

- Enrichment in Buffered *Listeria* Enrichment Buffer (BLEB)
- PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K and PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K
- MicroSEQ™ *Listeria* spp. Detection Kit
- 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: $\pm 1.6^{\circ}\text{C}/\text{sec}$ Fast mode: $\pm 3.5^{\circ}\text{C}/\text{sec}$
Thermal range	4-100°C
Thermal accuracy	$\pm 0.5^{\circ}\text{C}$
Thermal uniformity	$\pm 1^{\circ}\text{C}$
Format	96-well, 0.1-mL block

- Confirmation testing of positive samples as described in Chapter 5, “Recommended confirmation methods”.

Table 7 Validated matrices

Reference method	Matrix
ISO 11290–1:1996 with Amendment 1:2004	<ul style="list-style-type: none"> • Foods: pasteurized whole cow's milk, dry infant formula, roast beef, lox smoked salmon, hot dogs • Environmental surfaces: stainless steel, plastic cutting board, ceramic tile, rubber sheets, concrete sealed with Seal Hard™ sealant

Go to thermofisher.com/foodsafety for a list of workflows for detection of *Listeria* (Pub. No. MAN0009418).

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 DNAZap™ 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 22174:2005 or www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html.



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](https://www.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.



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:
www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 4th Edition, WHO/CDS/CSR/LYO/2020.12; found at:
www.who.int/publications/i/item/9789240011311



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](https://www.thermofisher.com/foodsafety)

Support email:

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

Phone: Visit [thermofisher.com/support](https://www.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](https://www.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
<i>RapidFinder™ Express Software Quick Reference</i>	4480999
<i>Thermo Scientific™ KingFisher™ Flex User Manual</i>	N07669

(continued)

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

References

ISO. 1996. Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Reference number ISO 11290:1:1996.

FDA Bacteriological Analytical Manual (BAM), Chapter 10 - Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods

