USER GUIDE

applied biosystems by life technologies™

MicroSEQ® *Listeria* Detection Kits

For detection of *L. monocytogenes* and *L.* spp.

Catalog Numbers 4403874, 4427410

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ALTERNATIVE ANALYTICAL METHODS
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TRADEMARKS:

Contents

About this guide

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

Revision history

Revision	Date	Description
A	November 2013	The following documents were combined to create this single user guide: • Pub. no. 4405962, *MicroSEQ® Listeria monocytogenes Detection Kit User Guide* • Pub. no. 4426510, *MicroSEQ® Listeria spp. Detection Kit User Guide*

1 Product information

Ensure that your instrument is properly installed and calibrated. For calibration information, see the documentation that is provided with your instrument.

Product description

The MicroSEQ® *Listeria* Detection Kits detect *Listeria* simply, reliably, and rapidly in food and environmental samples using a lyophilized reagent format. The assay is a single-well, real-time PCR in which *Listeria* DNA targets are amplified and detected in real time using fluorescent TaqMan® probes (hydrolysis probe chemistry). This kit is intended for investigators who need to test for *Listeria* in food and on environmental surfaces.

Kit	Specificity‡
MicroSEQ® *Listeria monocytogenes* Detection Kit	*Listeria monocytogenes*
MicroSEQ® *Listeria* spp. Detection Kit	All known species of *Listeria*

‡ For more details, see "Kit specificity" on page 38.

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

This protocol provides instructions for performing detection using:

Software	Chapter	Certification
RapidFinder™ Express software	2	AOAC‡ and AFNOR§
SDS software	3	None
StepOne™ software	4	None

‡ For more details, see "AOAC® Performance Tested Methodssm Certification" on page 39.
§ For more details, see "NF validation by AFNOR Certification" on page 40.

Kit contents and storage

Parts may ship separately depending on configuration and storage conditions.

Table 1 MicroSEQ® *Listeria monocytogenes* Detection Kit [96 reactions; Cat. no. 4403874]

Component	Description	Amount	Cap color	Storage‡
MicroSEQ® *Listeria monocytogenes* Detection Kit	*Listeria monocytogenes* Assay Beads, 8-tube strips	12 strips (96 tubes) 1 rack	Blue (rack)	5±3°C; Protect from light and moisture.§
	MicroAmp® Optical 8-Cap Strips	12 strips (96 caps)	N/A	
Pathogen Detection Negative Control	Pathogen Detection Negative Control	1.5 mL	Red	5±3°C.

‡ Refer to product label for expiration date.

§ 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.

Table 2 MicroSEQ® *Listeria* spp. Detection Kit [96 reactions; Cat. no. 4427410]

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

‡ Refer to product label for expiration date.

§ 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.

Materials required but not included in the kit

Note: A positive control is optional for the assay and needs to be made by the end-user. A positive control can be made by using a previously known positive sample or isolating nucleic acid from *Listeria* ordered from a commercial source, such as ATCC.

Unless otherwise indicated, all materials are available from Life Technologies.
MLS: major laboratory suppliers.

Item	Source‡
Instruments	
Applied Biosystems® 7500 Fast Real-Time PCR System	Contact your local Life Technologies sales office.
StepOne™ Real-Time PCR System	
StepOnePlus™ Real-Time PCR System	

Item	Source‡
Equipment	
Benchtop microcentrifuge with 8-tube strip adapter	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
(Optional but recommended) Plate centrifuge	MLS
Block heater	MLS
Laboratory mixer (Vortex mixer or equivalent)	MLS
MicroAmp® 96-Well Base	Cat. no. N8010531
MicroAmp® Cap Installing Tool	Cat. no. 4330015
7500 Fast Precision Plate Holder for MicroAmp® Tube Strips (for use with 7500 Fast Real-Time PCR System)	Cat. no. 4403809
MicroAmp® Fast 48-Well Tray (for use with StepOne™ Real-Time PCR System)	Cat. no. 4375282
MicroAmp® 96-Well Tray for VeriFlex™ Blocks (for use with StepOnePlus™ Real-Time PCR System)	Cat. no. 4379983
Consumables	
Aerosol-resistant pipette tips	MLS
Disposable gloves	MLS
MicroAmp® Fast 8-Tube Strip, 0.1-mL	Cat. no. 4358293
MicroAmp® Optical 8-Cap Strip, 300 strips	Cat. no. 4323032
Reagents	
For confirmation tests:	
PALCAM agar	MLS
Ottaviani & Agosti agar (OAA)	MLS
Nuclease-free Water	Cat. no. AM9938

‡ The materials listed here have been validated for use with this kit. Results may vary if substituted products from other vendors are used instead.

Recommended DNA isolation methods

Visit **www.lifetechnologies.com/foodsafety** for a list of workflows, including AOAC® and NF Validation (AFNOR) workflows, for detection of *L. monocytogenes* and *L.* spp.

Table 3 Recommended DNA isolation methods for use with the MicroSEQ® *Listeria* Detection Kits.

Nucleic acid isolation workflow	Kit	Cat. nos.	Resulting sample volume (µL)‡
Automated, magnetic bead-based	PrepSEQ® Nucleic Acid Extraction Kits§#	• 4428176 • 4480466	~140
Spin columns	PrepSEQ® Rapid Spin Sample Preparation Kits§	• 4426714 • 4426715‡‡	300
	PrepSEQ® Rapid Spin Sample Preparation Kits – Bead Beating#	• 4464654 • 4468304	300
Direct lysis	Lysis Buffer, FS *and* Proteinase K, FS	4480724 4480715	~200

‡ The *MicroSEQ® Listeria* Detection Kits require 30 µL of DNA sample added to each assay bead.
§ Part of AOAC® *Performance Tested Methods*℠ certification workflows.
# Part of NF Validation (AFNOR) certification workflows.
‡‡We recommend using the extra clean kits for matrices containing high fat content.

2 MicroSEQ® *Listeria* Detection Kits using RapidFinder™ Express software

Kit workflow

This chapter presents a protocol that uses RapidFinder™ Express software on the 7500 Fast Real-Time PCR System to automate MicroSEQ® *Listeria* Detection Kits processes.

Isolate DNA (page 12)

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MicroSEQ® Pathogen Detection Assay Using RapidFinder™ Express Software

Create or edit a run file using the RapidFinder™ Express software (page 14)

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Prepare the assay beads (page 14)
Set up the PCR (page 15)

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Load and run the instrument (page 16)

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View results and data analysis (page 17)

Important procedural guidelines

IMPORTANT! Seal the tubes with the transparent, optical strip-caps provided in the kit. Always use intact 8-cap strips, even if empty tubes have been added next to reaction tubes.

Do not use colored caps or tubes for real-time PCR. Colored caps or tubes may affect dye-signal readings during real-time PCR.

- RapidFinder™ Express Software determines the plate layout and therefore must be set up before distributing DNA samples for the real-time PCR.
- Cut the storage pouch above the zip-lock strip so that it can be resealed.
- Follow these additional tips when setting up the PCR:
  - 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.
  - 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 PCR practices" on page 37.

Create or edit a run file using the RapidFinder™ Express software

On the main page of the RapidFinder™ Express Software, select **Create/Edit a Run File**  , and enter 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. (If you are using RapidFinder™ Express Software v1.1, select **Pipette Samples**  on the main page for step-by-step instructions.)

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), one tube for each reaction.
2. Seal the storage pouch using the zip-lock strip, and store the pouch at 5±3°C.

   IMPORTANT! Do not remove the desiccant from the storage pouch.
3. If required by the software-determined 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

In the RapidFinder™ Express software, select **Pipette Samples** on the main page, select the appropriate run file, and follow the software prompts in the following procedure.

1. If necessary, thaw samples and controls completely.
2. To avoid cross-contamination, we recommend a brief centrifugation of all samples and controls to bring the contents to the bottom of the wells or tubes.
3. 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.
4. Seal the tubes with the transparent optical strip caps provided in the kit.

   Use the MicroAmp® 96-Well Base and the MicroAmp® Cap Installing Tool to avoid collapsing, bending, or misaligning the tubes. Confirm that the strips are straight and that each tube is in line with the adjacent tube.
5. Mark or label one end of the strip cap (but not directly on any cap) to maintain the strip orientation when transferring the tubes to the instrument tray.
6. Make sure reactions are thoroughly mixed:

   If reactions were not previously mixed during the pipetting step, place the assay tubes in a 96-well base and vortex to mix.
7. Make sure that the reagents are at the bottom of the tubes: briefly centrifuge the strip tubes at 200–600 × *g* for about 20 seconds using a centrifuge with a plate adapter or a bench top microcentrifuge with an 8-strip PCR tube adapter.

Load and run the instrument

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

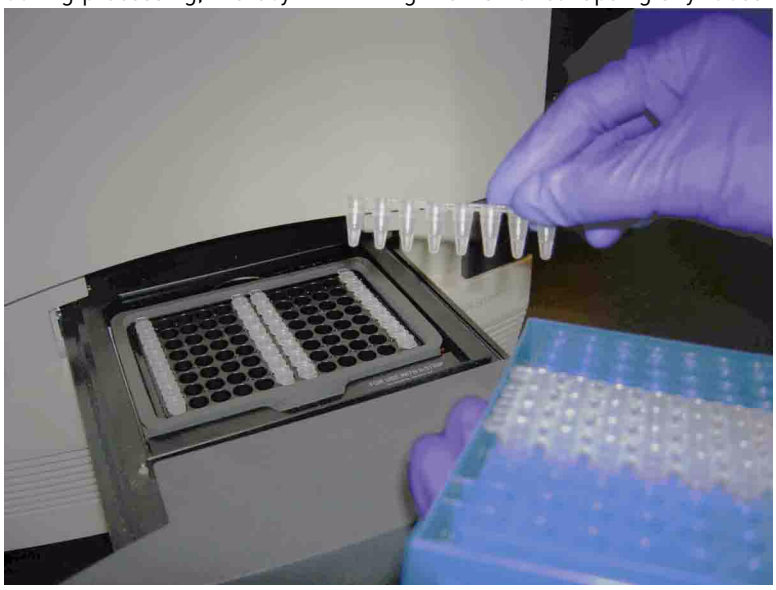
1. To load the instrument, transfer the tubes to the 7500 Fast system in the same configuration as the run layout.

   Use the 7500 Fast Precision Plate Holder for MicroAmp® Tube Strips in the instrument.

2. Close the tray for the instrument.

3. Follow the RapidFinder™ Express software prompts to start the run.

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



View results and data analysis

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

Data analysis is automated by the software.

If necessary, investigate results in the 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**, select and open the run file in RapidFinder™ Express Software, and then click **View in SDS**.
2. Select **File** ▶ **Save As**, then save the run file under a new name.

Confirmation of results

We do not recommend using the same method to screen samples and to confirm the results. When you use the MicroSEQ® Pathogen Detection System to screen samples, culture and biochemical methods are recommended to confirm the results.

The confirmatory methods to confirm positive results that can be used in the context of the AOAC® *Performance Tested Method*sm Certification and NF validation are described in "AOAC® Performance Tested Methodssm Certification" on page 39 and "NF validation by AFNOR Certification" on page 40, respectively.

3 MicroSEQ® *Listeria* Detection Kits using SDS software

Kit workflow

This chapter presents a protocol for using the *MicroSEQ® Listeria* Detection Kits with the SDS software on the 7500 Fast Real-Time PCR System.

Isolate DNA (page 12)

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MicroSEQ® Pathogen Detection Assay Using SDS Software

Prepare for PCR
- Create a run file document using SDS software (page 20)
- Prepare the assay beads (page 23)
- Prepare samples and controls (page 23)
- Prepare tubes for the 7500 Fast system (page 24)

▼

Start instrument run (page 25)

▼

View results on the 7500 Fast system (page 26)

Note: Any version of the SDS software can be used. However, see "General process using SDS software" on page 26 for the version and conditions that are used by the automatic data analysis done by RapidFinder Express™ software in certified workflows.

Important procedural guidelines

IMPORTANT! Seal the tubes with the transparent, optical strip-caps provided in the kit. Always use intact 8-cap strips, even if empty tubes have been added next to reaction tubes.

Do not use colored caps or tubes for real-time PCR. Colored caps or tubes may affect dye-signal readings during real-time PCR.

- Cut the storage pouch above the zip-lock strip so that it can be resealed.
- Follow these additional tips when setting up the PCR:
  - 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.
  - 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 PCR practices" on page 37.

Create a run file document using SDS software

1. In the SDS software  , create a run file document. Go to **File ▸ New** to open a New Document Wizard.
2. In the Define Document section of the New Document Wizard:

For...	Select...
Assay	Absolute Quantitation (Standard Curve)
Container	96-Well Clear
Template	Blank Document
Run mode	7500 Fast
For all other fields, enter the requested information and click **Next** to continue.	

Note: For more information on creating a run file document, refer to the documentation that is provided with your instrument.

3. In the Select Detectors section of the New Document Wizard:
   a. Select detectors that contain the following characteristics:

      Reporters for **FAM** and for **VIC**, each with the **Quenchers** set at **none**.

      The FAM™ dye is used to detect the targets; the VIC® dye is used to detect the internal positive control (IPC).
   b. Click **Add** to enter the detector into the **Detectors in Document** field.
   c. Click **Next** to continue.

Note: If a detector is missing from the list, then click **New Detector** and create a new detector for each reporter dye. Select a different color for each reporter dye.

4. In the Set Up Sample plate section of the New Document Wizard:
   a. Assign the FAM™ and VIC® reporter dyes to each well that contains sample.
   b. Click on the **Use** checkbox next to each detector.
   c. Click **Finish** to continue.

   If needed, review "Start instrument run" on page 25 to determine the optimal layout to minimize bending or misaligning the tube strips.

   Note: If FAM™ and VIC® detectors are missing from the list of detectors, use the **Back** tab to add the missing detectors to the "Detectors in Document" field.

   Each well that contains sample should contain two "U" symbols that correspond to the FAM™ and VIC® reporter dyes.

5. Under the Setup tab, enter sample names for each sample by double-clicking the sample well.

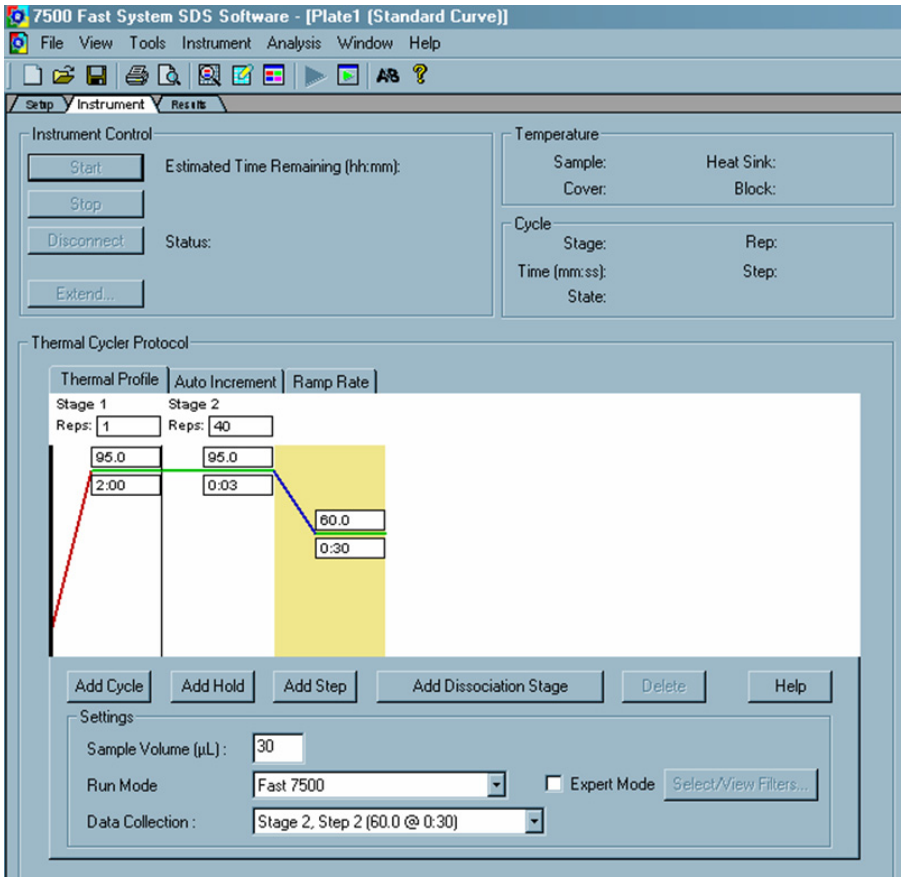
6. Under the Instrument tab, set thermal cycling conditions as indicated in the following table. For more details, refer to the *7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide* (Pub. no. 4347825).

Step	Enzyme activation	PCR	
	HOLD	Cycle (40 cycles)	
		Denature	Anneal/extend
Temp.	95°C	95°C	60°C
Time	2 min	3 sec	30 sec

7. Set the Sample Volume to **30 µL**.

8. Under File, save the run file as a *.sds* document in the appropriate folder.

Figure 1 7500 Fast system with SDS software: thermal cycling conditions for run mode **Fast 7500**



Prepare the assay beads

1. Transfer the appropriate number of individual tubes or 8-tube strips from the pouch, based on the number of samples and controls that you plan to run, to a 96-well base at room temperature (23±5°C).

   At least one negative control is recommended for the target pathogen.

   Remove colored caps and discard. Avoid disturbing the beads from the bottom of the tubes.

2. Seal the storage pouch using the zip-lock strip, then store at 5±3°C.

IMPORTANT! Do not remove the desiccant from the storage pouch.

Prepare samples and controls

1. Thaw all reagents (samples and controls) completely.
2. To avoid cross-contamination, we recommend a brief centrifugation of all samples and controls to bring the contents to the bottom of the wells or tubes.
3. Add 30 µL of each sample or control prepared above to each assay bead at room temperature (23±5°C).

   Dispense all unknown samples first, followed by negative control(s) and then positive control(s).

4. Mix by gently aspirating and dispensing a few times. (Alternatively, vortex assay tubes after they are capped in the final step.)

   Beads dissolve in 1–5 seconds.

Prepare tubes for the 7500 Fast system

1. For 8-tube strips with seven or fewer reactions, add additional empty tubes as needed so that each strip contains a full set of 8 tubes.

   Note: The empty capped 8-tube strips evenly distribute the clamping load that is applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes.

2. Seal the tubes with the transparent, optical strip caps provided in the kit, and confirm that the strips are straight and that each tube is in line with the adjacent tube.

   Use the MicroAmp® 96-Well Base and the MicroAmp® Cap Installing Tool to avoid collapsing, bending, or misaligning the tubes.

3. Mark or label one end of the strip cap (but not over a cap) to maintain strip orientation when transferring the tubes to the instrument tray.

4. Make sure reactions are thoroughly mixed:

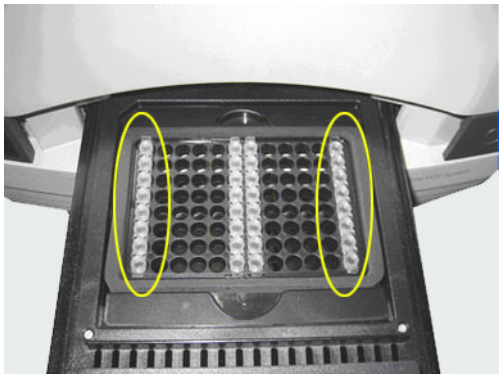
   If reactions were not previously mixed during the pipetting step, place the assay tubes in a 96-well base and use a vortexer to mix.

5. Make sure that the reagents are at the bottom of the tubes: briefly centrifuge the strip tubes at 200–600 × *g* for approximately 20 seconds using a centrifuge with a plate adapter or a bench top microcentrifuge with an 8-strip PCR tube adapter.

Start instrument run

Use the 7500 Fast Precision Plate Holder for MicroAmp® Tube Strips in the instrument.

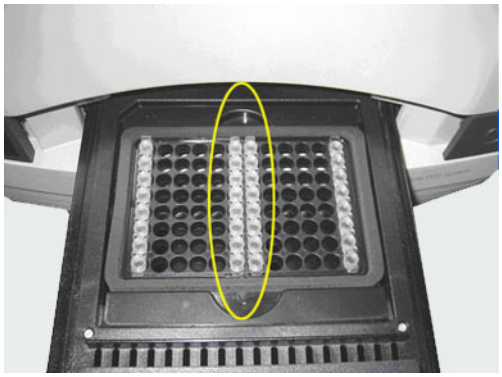
1. (Optional) If column 1 (leftmost) and column 12 (rightmost) are not used, insert two fully capped, empty, 8-tube strips into these columns. The empty capped 8-tube strips evenly distribute the clamping load applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes.



2. Carefully insert two or more 8-tube strips containing samples, starting from the center of the plate holder and moving out. This layout minimizes bending or misaligning the tube strips.

   Note: A minimum of two and a maximum of twelve 8-tube strips can be run at one time.

IMPORTANT! Always use a total of 8 tubes per column. You may need to add new, empty tubes to a column.



3. Open the run file that you created in "Create a run file document using SDS software" on page 20.

4. In the Instrument tab, select **Start** to begin the run.

IMPORTANT! To avoid false positives due to amplified material in your work area, do not open tubes after amplification.

View results

General process using SDS software

The general process for viewing results generated using the MicroSEQ® *Listeria* Detection Kits involves the following:

- View the amplification plots for all reactions.
- Set the baseline and threshold values. For example, the following version and conditions are used by the automatic data analysis done by RapidFinder Express™ software in certified workflows:

Software or parameter	AOAC®-certified workflow	NF validation by AFNOR certification workflow
SDS software	Version 1.4	Version 1.4
Auto or Manual C_t setting	Manual C_t	Manual C_t
Threshold values	• *L. mono*: 0.5 • *L.* spp.: 0.65 • IPC: 0.3	• *L. mono*: 0.5 • *L.* spp.: 0.5 • IPC: 0.3
Baseline	Autobaseline	Autobaseline
Positive cut off values‡	• *L. mono*: 35.0 • *L.* spp.: 35.027 • IPC: 40.0	• *L. mono*: 36.7 • *L.* spp.: 38.72 • IPC: 40.0

‡ When using the recommended instrument threshold and baseline settings.

- Check each sample for FAM™ dye (target) and VIC® dye (IPC) signal. The following table is a basic guide for interpreting the results:

FAM™ dye signal (target)	VIC® dye signal (IPC)	Results
+	+	Positive
+	–	Positive
–	+	Negative
–	–	Inconclusive; no IPC detected and no target-specific signal detected

Confirmation of results

We do not recommend using the same method to screen samples and to confirm the results. When you use the MicroSEQ® Pathogen Detection System to screen samples, culture and biochemical methods are recommended to confirm the results.

4 MicroSEQ® *Listeria* Detection Kits using StepOne™ software

Kit workflow

This chapter presents a protocol for using the MicroSEQ® *Listeria* Detection Kits with the StepOne™ software on the StepOne™ and StepOnePlus™ instruments.

Isolate DNA (page 12)

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MicroSEQ® Pathogen Detection Assay Using StepOne™ Software

Prepare for PCR

- Create a run file document using StepOne™ software (page 28)
- Prepare the assay beads (page 30)
- Prepare samples and controls (page 31)
- Prepare tubes for the StepOne™ and StepOnePlus™ Systems (page 31)

▼

Start instrument run

- Run the 8-tube reactions on the StepOne™ System (page 32)
- Run the 8-tube reactions on the StepOnePlus™ System (page 33)

▼

View results on the StepOne™ and StepOnePlus™ Systems (page 34)

Note: The StepOne™ and StepOnePlus™ instruments were not included in either the AOAC® or NF validation studies.

Important procedural guidelines

IMPORTANT! Seal the tubes with the transparent, optical strip-caps provided in the kit. Always use intact 8-cap strips, even if empty tubes have been added next to reaction tubes.

Do not use colored caps or tubes for real-time PCR. Colored caps or tubes may affect dye-signal readings during real-time PCR.

- Cut the storage pouch above the zip-lock strip so that it can be resealed.
- Follow these additional tips when setting up the PCR:
  - 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.
  - 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 PCR practices" on page 37.

Create a run file document using the StepOne™ software

1. In the StepOne™ software, create a run file document.

   Go to **File ▸ New Experiment** and select **Advanced Setup**.

   Note: For information on creating a run file document, refer to the documentation that is provided with your instrument.

2. In the Experiment Properties page:

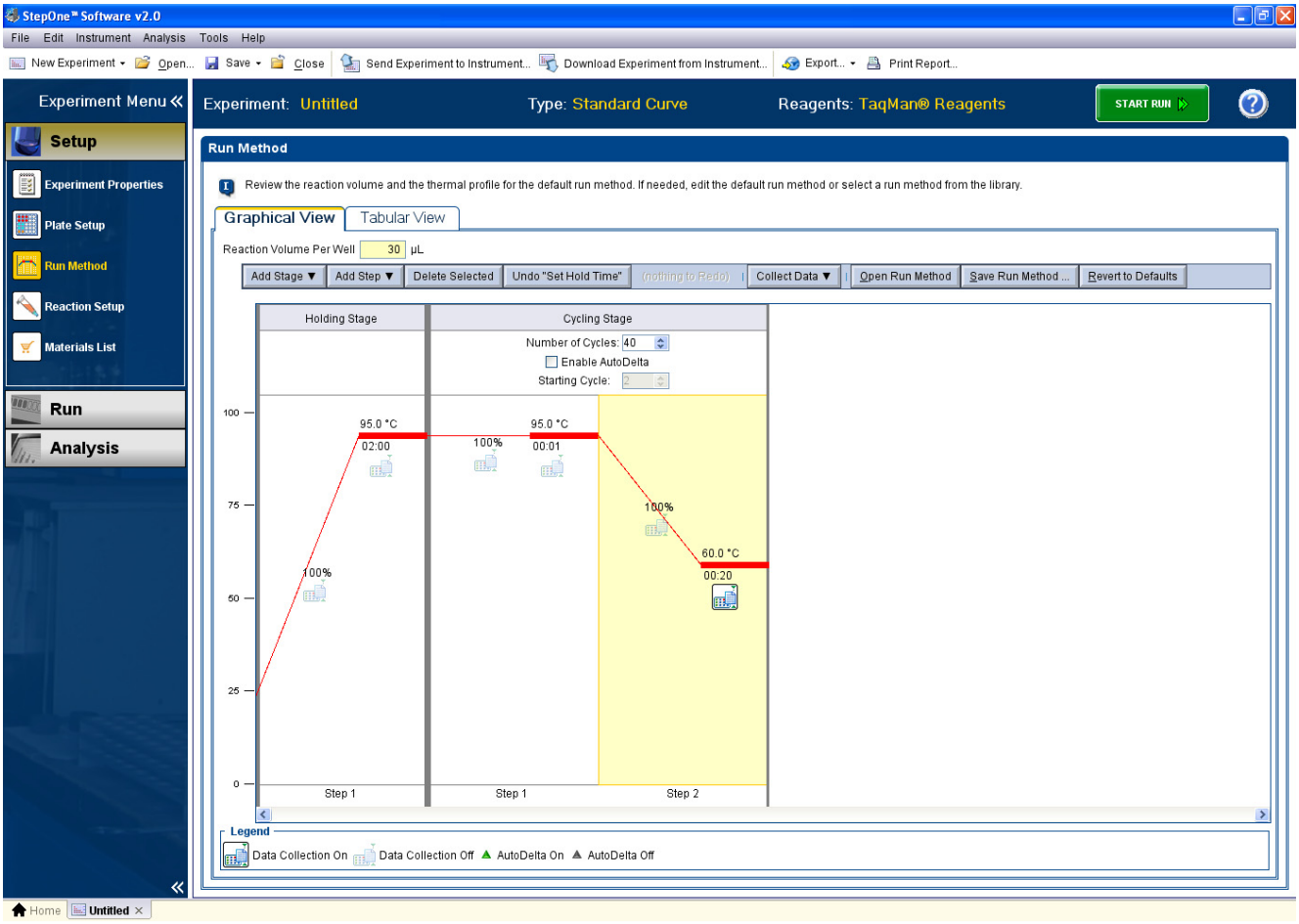
For...	Select...
Instrument	StepOne™ Instrument – (48 Wells) *or* StepOnePlus™ Instrument – (96 Wells)
Type of experiment	Quantitation – Standard Curve
Type of reagents	TaqMan® Reagents
Ramp speed	Fast

3. In the Plate Setup page, under the Define Targets and Samples tab:
   a. Create two targets with NFQ-MGB quenchers.
   b. Select FAM™ and VIC® as the reporter dyes.
      The FAM™ dye is used to detect the targets; the VIC® dye is used to detect the internal positive control (IPC).
4. Under the Assign Targets and Samples tab, associate FAM™ and VIC® detectors with each reaction.
   If needed, review "Run the 8-tube reactions on the StepOne™ System" on page 32 or "Run the 8-tube reactions on the StepOnePlus™ System" on page 33, as appropriate, to determine the optimal layout in order to minimize bending or misaligning the tube strips.
5. In the Run Method page, set thermal cycling conditions as indicated in the following table. For more details, refer to "Chapter 5 Presence/Absence Experiments" in the *StepOne™ and StepOnePlus™ Real-Time PCR Systems User Guide* (Pub. no. 4379704).

Step	**Enzyme activation**	**PCR**	
	HOLD	Cycle (40 cycles)	
		Denature	Anneal/extend
Temp.	95°C	95°C	60°C
Time	2 min	1 sec	20 sec

6. Set the Sample Volume to **30** µL.
7. Under File, save the run file as a *.eds* document in the appropriate folder.

Figure 1 StepOnePlus™ instrument using StepOne™ software: thermal cycling conditions with fast ramping.



Prepare the assay beads

1. Transfer the appropriate number of individual tubes or 8-tube strips, based on the number of samples and controls that you plan to run, to a 96-well base at room temperature (23±5°C).

   At least one negative control is recommended for the target pathogen.

   Remove colored caps and discard. Avoid disturbing the beads from the bottom of the tubes.

2. Seal the storage pouch using the zip-lock strip, then store at 5±3°C.

IMPORTANT! Do not remove the desiccant from the storage pouch.

Prepare samples and controls

1. Thaw all reagents (samples and controls) completely.
2. To avoid cross-contamination, we recommend a brief centrifugation of all samples and controls to bring the contents to the bottom of the wells or tubes.
3. Add 30 µL of sample or control prepared above to each assay bead at room temperature (23±5°C).

   Dispense all unknown samples first, followed by negative control(s) and then positive control(s).
4. Mix by gently aspirating and dispensing a few times. (Alternatively, vortex assay tubes after they are capped in the final step.)

   Beads dissolve in 1–5 seconds.

Prepare tubes for the StepOne™ and StepOnePlus™ Systems

1. For 8-tube strips with seven or fewer reactions, add additional empty tubes as needed so that each strip contains a full set of 8 tubes.

   Note: The empty capped 8-tube strips evenly distribute the clamping load that is applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes.
2. Seal the tubes with the transparent, optical strip caps provided in the kit, and confirm that the strips are straight and that each tube is in line with the adjacent tube.

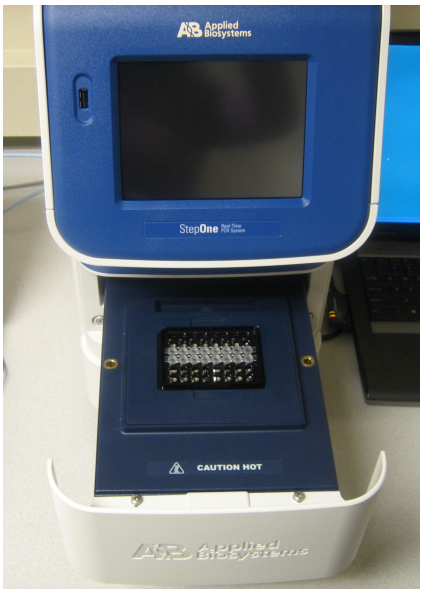
   Use the MicroAmp® 96-Well Base and the MicroAmp® Cap Installing Tool to avoid collapsing, bending, or misaligning the tubes.
3. Mark or label one end of the strip cap (but not over a cap) to maintain strip orientation when transferring the tubes to the instrument tray.
4. Make sure reactions are thoroughly mixed:

   If reactions were not previously mixed during the pipetting step, place the assay tubes in a 96-well base and use a vortexer to mix.
5. Make sure that the reagents are at the bottom of the tubes: briefly centrifuge the strip tubes at 200–600 × *g* for approximately 20 seconds using a centrifuge with a plate adapter or a bench top microcentrifuge with an 8-strip PCR tube adapter.

Start instrument run

This section describes the procedure for running the 8-tube reactions on the StepOne™ (page 32) and StepOnePlus™ (page 33) Real-Time PCR Systems using StepOne™ software.

Run the 8-tube reactions on the StepOne™ System



1. Place the MicroAmp® Fast 48-Well Tray on the sample block of the StepOne™ System.
2. Load the 8-tube strips horizontally (see figure above).

   For example, in Row C, load an 8-tube strip across columns 1 through 8.

   A minimum of one 8-tube strip is recommended. It is not necessary to balance the tube strips on the tray.
3. Open the run file document that corresponds to the reaction plate that you created in "Create a run file document using the StepOne™ software" on page 28.
4. Start the run.

IMPORTANT! To avoid false positives due to amplified material in your work area, do not open tubes after amplification.

Run the 8-tube reactions on the StepOnePlus™ System

1. Place the MicroAmp® 96-Well Tray for VeriFlex™ Blocks on the sample block of the StepOnePlus™ System.
2. Load the 8-tube strips vertically (see figure above).

   The minimum recommended load is two 8-tube strips (16 tubes), which you should place in adjacent columns, for example in columns 1 and 2. It is not necessary to balance the tube strips on the tray.
3. Open the run file document that corresponds to the reaction plate that you created in "Create a run file document using the StepOne™ software" on page 28.
4. Start the run.

IMPORTANT! To avoid false positives due to amplified material in your work area, do not open tubes after amplification.

View results

General process using StepOne™ software

The general process for viewing results generated using the MicroSEQ® *Listeria* Detection Kits involves the following:

- View the amplification plots for all reactions.
- Set the baseline and threshold values.
- Check each sample for FAM™ dye (target) and VIC® dye (IPC) signal. The following table is a basic guide for interpreting the results:

FAM™ dye signal (target)	VIC® dye signal (IPC)	Results
+	+	Positive
+	–	Positive
–	+	Negative
–	–	Inconclusive; no IPC detected and no target-specific signal detected

Confirmation of results

We do not recommend using the same method to screen samples and to confirm the results. When you use the MicroSEQ® Pathogen Detection System to screen samples, culture and biochemical methods are recommended to confirm the results.

A Troubleshooting

Observation	Possible cause	Recommended action
In positive-control wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.	No action is required. The result is considered positive.
In positive-control wells, no target-specific signal is detected.	Positive control was omitted (pipetting error).	Repeat the assay. Make sure to pipet positive control into all positive-control wells.
In negative-control wells, no IPC is detected, but target-specific signal is detected.	• Carryover contamination caused target signal in negative-control wells. • 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 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-control wells, target-specific signal is detected.	Carryover contamination occurred.	1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 2. If the negative control continues to show contamination, repeat the assay using a new kit. 3. If the negative control continues to show contamination, contact Applied Biosystems Technical Support.
In unknown sample wells, no IPC or target-specific signal is detected.	Inhibition of PCR occurred.	Dilute the sample 1:5 or 1:10 with nuclease-free water to dilute PCR inhibitors, and repeat the assay. If the PCR remains inhibited, repeat the sample preparation. For other suggestions, refer to the "Troubleshooting" section in your PrepSEQ® user guide.
In unknown sample wells, no IPC is detected, but target-specific signal (C_t <35) 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
Multicomponent Plot signals for FAM™, VIC®, and ROX™ dyes increase/decreases during cycles 1–15, but the overall curve and result is not affected (this observation applies to **View in SDS** mode).	Incomplete mixing and dissolution of the lyophilized bead with sample or control.	After addition of 30 µL of sample or Pathogen Negative Control to the beads and capping the tubes: 1. Vortex strips at high speed for about 10 seconds and centrifuge the strips at 200–600 × *g* for about 10 seconds. 2. Vortex the strips again at high speed for about 10 seconds, and centrifuge the strips at 200–600 × *g* for about 1 minute. Ensure that all liquid is at the bottom of the tubes and the beads are fully dissolved before proceeding with the PCR.
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 tubes.	Prepare negative control samples using at least one 8-tube strip of MicroSEQ® Assay Beads. 1. Divide the number of assay beads into 2 sets. a. To the first set of assay beads, add 30 µL of nuclease-free water. b. To the second set of assay beads, add 29 µL of nuclease-free water plus 1 µL of 1 U/µL Uracil DNA Glycosylase (Cat. no. 18054-015). 2. Run samples on the 7500 Fast Real-Time PCR Instrument using SDS software and select the **Fast 7500** Run Mode. 3. Under the instrument tab: • Select **Add Step** to stage 1 of the PCR cycle that consists of 10 minutes at 50°C. • Extend the 95°C step from 20 seconds to 10 minutes. Amplicon contamination is indicated by target-specific signal in the –UNG samples and no target-specific signal in +UNG samples. If the instrument block was contaminated, consult the *7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide* (Pub. no. 4347825) and/or contact a service representative to clean the instrument.

B

Good PCR practices

Prevent contamination and nonspecific amplification

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.

PCR good laboratory practices

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR 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 pipette or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA*Zap*™ Solutions (Cat. no. AM9890).
- To avoid false positives due to cross-contamination:
  - Prepare and close all negative-control and unknown sample tubes before pipetting the positive control.
  - Do not open tubes after amplification.
  - Use different sets of pipettors when pipetting negative-control, unknown, and positive-control samples.

Plate layout suggestions

- Separate different targets by a row if enough space is available.
- Put at least one well between unknown samples and controls if possible.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- Put positive controls in one of the outer rows or columns if possible.
- Consider that caps come in strips of 8 or 12.

C

Background information

Product overview

Description of target microorganisms

The genus Listeria is composed of six species; *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii*, *L. grayi* and two recently discovered new species, given the provisional names *L. marthii* and *L. rocourti*. *L. monocytogenes* is of clinical relevance for humans because it is the causative agent for Listeriosis. *Listeria monocytogenes* poses a severe risk to pregnant women, potentially causing spontaneous abortion or stillbirth. Normally, *L. monocytogenes* is transferred to humans through raw milk, soft-ripened cheeses, raw vegetables, poultry, raw meats, and raw or smoked fish. *L. monocytogenes* grows at temperatures as low as 3°C, allowing it to multiply in refrigerated foods.

Kit sensitivity

The sensitivity of the assay in culture samples depends on the quality of the sample preparation method that is used. The AOAC® *Performance Tested Methods*℠ workflow described in this user guide allows you to detect 1 to 3 colony-forming units (CFU) from 25 grams of food or from environmental swab and sponge samples after enrichment and sample preparation with one of the following kits:

- PrepSEQ® Nucleic Acid Extraction Kit
- PrepSEQ® Rapid Spin Sample Preparation Kit

Refer to *Workflows for Detection of Listeria in Food and Environmental Samples* to choose an appropriate kit for your laboratory (Pub. no. MAN0009418, available at **www.lifetechnologies.com/foodsafety**).

Kit specificity

The MicroSEQ® *Listeria monocytogenes* Detection Kit can detect the following serotypes: 1/2A, 1/2B, 1/2C, 3A, 3B, 3C, 4A, 4AB, 4B, 4C, 4D, 4E, and 7. The kit does not detect other *Listeria* species or non-*Listeria* pathogens.

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*.

Note: When using enrichments in half and whole Fraser broth in the PrepSEQ® Nucleic Acid Extraction Kit workflow with automated sample preparation, *L. grayi* is not detected due to its poor growth in whole Fraser broth.

Operational conditions

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

Table 1 Temperature and humidity requirements

Condition	Acceptable range
Temperature	15 to 30°C (50 to 90°F) Maximum change of less than 15°C (59°F) per 24 hours
Humidity	20 to 80% relative humidity, noncondensing

AOAC® *Performance Tested Methods*℠ Certification

Visit **www.lifetechnologies.com/foodsafety** for a list of workflows for the detection of *L. monocytogenes* and *L.* spp.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 011002

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 021108

Workflow

The *MicroSEQ® Listeria* Detection Kits earned the *Performance Tested Methods*℠ Certification from the AOAC® Research Institute. The validated workflow includes:

- Enrichment media: Buffered *Listeria* Enrichment Buffer (BLEB)
- Sample preparation kit options:
  - PrepSEQ® Nucleic Acid Extraction Kit
  - PrepSEQ® Rapid Spin Sample Preparation Kits

  Refer to *Workflows for Detection of Listeria in Food and Environmental Samples* to choose an appropriate user guide (Pub. no. MAN0009418, available at **www.lifetechnologies.com/foodsafety**).
- *MicroSEQ® Listeria* Detection Kits

Kit	AOAC® Research Institute License Number
MicroSEQ® *Listeria monocytogenes* Detection Kit	011002
MicroSEQ® *Listeria* spp. Detection Kit	021108

- Applied Biosystems® 7500 Fast Real-Time PCR System
- RapidFinder™ Express Software
- Confirmation testing of positive samples

In the context of AOAC® Validation, when BLEB is used for enrichment media, as shown in the AOAC®-validated workflow, you can refer to the USFDA Bacteriological Analytical Manual (BAM), Chapter 10; see **www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm** and scroll to *Listeria monocytogenes*.

Matrices

The workflow was certified for use with the following matrices:

License number (target organisms)	Reference method	Matrix
011002 (*l. monocytogenes*)	ISO 11290–1:1996 with Amendment 1:2004	**Foods**: roast beef, cured bacon, lox, lettuce, pasteurized whole cow's milk, dry infant formula, ice cream, salad dressing, mayonnaise
021108 (*l.* spp.)	ISO 11290–1:1996 with Amendment 1:2004	**Foods**: roast beef, hot dogs, lox, pasteurized whole cow's milk, dry infant formula **Environmental surfaces**: stainless steel, plastic cutting board, ceramic tile, rubber sheets, concrete sealed with Seal Hard® sealant

Confirmation of results

In terms of AOAC® validation, enriched cultures with positive PCR results were tested further by cultural confirmation following ISO 11290–1.

NF validation by AFNOR Certification

Visit **www.lifetechnologies.com/foodsafety** for a list of workflows for the detection of *L. monocytogenes* and *L.* spp.

Certification	Expiration
BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140 **ABI 29/05 – 12/11** **ABI 29/04 – 12/11** ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS **www.afnor-validation.com**	For information about the expiration date of the NF Validation certification, refer to the certificates, ABI 29/05 – 12/11 and ABI 29/04 – 12/11, available at **www.afnor-validation.com**.

Workflow

The *MicroSEQ® Listeria* Detection Kits have been certified "NF Validation". The certification uses the ISO 16140 standard for the validation of alternative methods (Alternative Analytical Methods for Agribusiness. Certified NF Validation; **www.afnor-validation.com**). This kit was compared and found equivalent to the ISO 11290-1 reference method. The validated workflow includes:

- Enrichment:

Kit	Media	Conditions
PrepSEQ® Nucleic Acid Extraction Kit	1. Half Fraser Broth (primary enrichment) 2. Fraser Broth (secondary enrichment)	1. 30±1°C for 24–28 hours 2. 37±1°C for 16–24 hours
PrepSEQ® Rapid Spin Sample Prep Kit	Half Fraser Broth	37±1°C for 24–32 hours

- Optional storage of enriched cultures at 5±3°C for up to 72 hours prior to DNA preparation
- Sample preparation kit options:
  - PrepSEQ® Nucleic Acid Extraction Kit for Food and Environmental Testing
  - PrepSEQ® Rapid Spin Sample Prep Kit – Bead Beating
  - PrepSEQ® Rapid Spin Sample Prep Kit – Extra Clean and Bead Beating (for meat samples)

  Refer to *Workflows for Detection of Listeria in Food and Environmental Samples* to choose an appropriate user guide (Pub. no. MAN0009418, available at **www.lifetechnologies.com/foodsafety**).
- *MicroSEQ® Listeria* Detection Kits:

Kit	Certificate
MicroSEQ® *Listeria monocytogenes* Detection Kit	ABI 29/05 - 12/11
MicroSEQ® *Listeria* spp. Detection Kit	ABI 29/04 - 12/11

- Applied Biosystems® 7500 Fast Real-Time PCR System
- RapidFinder™ Express Software
- Confirmation testing as described in "Confirmation of results" on page 42

Matrices

This workflow has been validated for use with the following matrices:

Reference method	Matrix
ISO 11290-1	All food and feed categories and environmental samples • Meat products (processed and unprocessed): Poultry, pork, and beef • Dairy products: Milks and fermented milks, raw milk cheeses, desserts, and ice cream • Seafood: Raw fish, smoked and cured fish, ready-to-eat foods • Vegetables: Fresh and frozen vegetables, spices, aromatic herbs, ready-to-eat foods • Environmental samples: Swabs, dust, cleaning and process water

Please note that the method of using an automated extraction protocol (that is, the PrepSEQ® Nucleic Acid Extraction Kit) with the MicroSEQ® *Listeria* spp. Detection Kit does not allow for the detection of *Listeria grayi* due to poor growth in whole Fraser broth.

Confirmation of results

In the context of NF Validation: all samples identified as positive by the MicroSEQ® *Listeria monocytogenes* Detection Kit or the MicroSEQ® *Listeria* spp. Detection Kit must be confirmed by any of the following means:

- When Half Fraser Broth is used for enrichment media, as shown in the workflow validated by AFNOR certification, streak 100 µL of enrichment broth on OAA agar. If necessary, subcultures can be performed in Fraser Broth before streaking (10 µL) on OAA agar. Characteristic colonies can be further confirmed by classical biochemical tests.
- Test as described in the NF EN ISO 11290-1 reference method
- Any other method certified "NF Validation" based on a different principle than the MicroSEQ® *Listeria monocytogenes* Detection Kit or the MicroSEQ® *Listeria* 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 (positive with the alternative method, unconfirmed by one of the means described above), the laboratory must follow the necessary steps to guarantee the validity of the obtained result. If a positive PCR result cannot be confirmed by streaking 100 µL of culture on PALCAM or OAA agar, look at the SDS file and note the C_t value from the specific PCR well. If the well has a FAM dye C_t value >34, we recommend repeating the sample extraction. In rare cases, high C_t values can be the result of cross contamination, in which case, the second extraction should turn out negative.

General recommendations

- We recommend complying with Good Laboratory Practices (GLP; refer to EN ISO 7218 standard).
- We recommend that ISO 11290-1 and ISO 6887 be followed for the preparation of initial suspensions.
- In the context of NF Validation, samples of more than 25 g have not been tested.

D

Safety

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

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

Chemical safety

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

- Read and understand the 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.

Specific chemical handling

CAS	Chemical information	
26628-22-8	Sodium Azide	Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Biological hazard safety

WARNING! *Listeria monocytogenes* and other *Listeria* spp. are Biosafety Level 2 (BSL-2) organisms. Pregnant women or immuno-compromised individuals, in particular, should understand the severe, potential risks associated with working with *Listeria monocytogenes*. Care must be taken when handling samples that may contain *Listeria* spp. Laboratory personnel must be adequately trained to handle pathogens before being permitted to analyze samples for *Listeria* spp. Laboratory personnel must wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. Waste should be disposed of in compliance with local and national legislation as appropriate.

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. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

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

References

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

ISO. 1999–2003. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Reference number 6887.

ISO. 2007. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations. Reference number 7218:2007.

ISO. 2003. Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods. Reference number 16140:2003.

2010. Association of Analytical Chemists (AOAC). **www.aoac.org**. Accessed November 2010.

2012. NF Validation by AFNOR-Certification. **www.afnor-validation.com**. Accessed August 2012.

USFDA Bacteriological Analytical Manual (BAM), Chapter 10; go to **www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm** and scroll to *Listeria monocytogenes.*

Documentation and support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Food safety support

Website: **www.lifetechnologies.com/foodsafety**

Support email: foodsafety@lifetech.com

Phone number (In North America): 1-800-500-6885

Phone number (Outside of North America): Go to **www.lifetechnologies.com/contactus.html** and select the appropriate country from the drop-down menu.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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