USER GUIDE

applied biosystems by life technologies™

PrepSEQ® Rapid Spin Bead-Beating Sample Prep Kit: *Listeria* spp. and *L. monocytogenes*

Catalog Numbers 4464654, 4468304 (Extra Clean)

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ALTERNATIVE ANALYTICAL METHODS
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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
B	December 2013	• Updated workflow schematic with more details from the protocol section • Clarified the enrichment media included in the NF Validation™ certification studies • Added an About this guide section • Updated legal and safety statements as needed to be in keeping with the latest Life Technologies document templates
A	October 2013	New user guide

Product information

Product description

The PrepSEQ® Rapid Spin Bead-Beating Sample Prep Kits are designed for preparation of food and environmental samples for testing for *Listeria species or Listeria monocytogenes* by real-time PCR.

This guide provides instructions for:

- Enrichment of food and environmental samples for *Listeria*.
- Preparation of DNA for PCR from 750 µL of enriched sample using one of the following kits:
  - **PrepSEQ® Rapid Spin Sample Prep Kit – Bead Beating** (Cat. no. 4464654).
  - **PrepSEQ® Rapid Spin Sample Prep Kit – Extra Clean & Bead Beating** (Cat. no. 4468304).

Choose a kit and protocol according to the following table.

Sample type	Recommended kit	Recommended protocol
• Food samples with little or no fat, such as vegetables, juices, and lean meats (less than 10% fat content) • Environmental samples (swab or sponge)	PrepSEQ® Rapid Spin Sample Prep Kit – Bead Beating	Standard protocol (page 14)
• Food samples containing high-fat content, such as cheese, milk, infant formula, smoked salmon (lox), chicken wing samples, and many meat products • Samples in which inhibition of PCR is frequently observed	PrepSEQ® Rapid Spin Sample Prep Kit – Extra Clean & Bead Beating	Extra-clean protocol (page 16)

Refer to Appendix A on page 21 for information about the NF validation™ workflows.

Note: During the NF Validation™ studies, all meats were treated with the extra-clean protocol.

Procedure overview

The procedure workflow is shown on page 11.

First, food or environmental samples are enriched for *Listeria* as appropriate to the sample matrix.

In the standard protocol, 750 µL of enriched food or environmental sample is filtered through the spin column into a zirconium bead-containing tube. During this step, the bacteria flow through the column while larger particulates are trapped in the column. A proprietary black coloring agent binds to the bacteria to make the pellet easily visible throughout the procedure. The zirconium bead/bacterial pellet is washed with Nuclease-free Water, then resuspended in Lysis Buffer. The bacteria are lysed by bead-beating, followed by incubation at 97 ±2°C. The lysate is diluted with Nuclease-free Water, and centrifuged to pellet remaining debris and the coloring agent. The nucleic acid remains in the supernatant.

In the extra-clean protocol, 750 µL of enriched food sample is first filtered through the spin column into a clean tube *without* zirconium beads, to allow binding of the black coloring agent to the bacteria in the sample. The (black) bacterial pellet is resuspended in Nuclease-free Water and transferred to a zirconium bead-containing tube. This transfer step helps to remove excess oil and fat that may have accumulated in the original tube. The zirconium bead/bacteria mixture is centrifuged and the bead/bacterial pellet is resuspended in Lysis Buffer and processed as in the standard protocol.

The final supernatant, containing the sample DNA, is ready for downstream PCR using the MicroSEQ® *Listeria monocytogenes* Detection Kit (Cat. no. 4403874) or the MicroSEQ® *Listeria* spp. Detection Kit (Cat. no. 4427410), or it can be stored as described in "DNA sample storage before real-time PCR" on page 18.

Kit contents

Each kit contains reagents for 100 sample preparations.

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

PrepSEQ® Rapid Spin Sample Prep Kit – Bead Beating (Cat. no. 4464654)

Component	Quantity	Storage
Spin columns	100	Room temperature (23±5°C)
Microcentrifuge tubes, zirconium beads, 1.5 mL	100	Room temperature (23±5°C)
Lysis Buffer, 1 bottle	5 mL	5±3°C

PrepSEQ® Rapid Spin Sample Prep Kit – Extra Clean & Bead Beating (Cat. no. 4468304)

Item	Quantity	Storage
Spin columns	100	Room temperature (23±5°C)
Microcentrifuge tubes, 1.5 mL	100	Room temperature (23±5°C)
Microcentrifuge tubes, zirconium beads, 1.5 mL	100	Room temperature (23±5°C)
Lysis Buffer, 1 bottle	5 mL	5±3°C

Required materials not included in the kits

Unless otherwise indicated, many of the listed items are available from major laboratory suppliers (MLS).

Note: The materials listed here have been validated for use with this kit. Results may vary if alternate products from other vendors are used.

Item	Source
Equipment	
Block heater, 95°C	MLS
Rack for 1.5-mL tubes	MLS
Benchtop microcentrifuge	Eppendorf 5415D or equivalent
Homogenizer (Stomacher® 400 Laboratory Blender)	Seward # 0400/001/AJ or equivalent
Vortex-Genie® Vortex Mixer	MLS
Vortex Adaptor (holds 12 tubes)	Life Technologies Cat. no. AM10024
Pipettors: • Positive-displacement • Air-displacement	MLS
Consumables	
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS
15-mL conical tubes	MLS
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 Blender, or equivalent)	Nasco # B01348WA, or equivalent
Homogenizer bag, no mesh, 6" × 9", 24 oz, (Whirl-Pak® Write-on Bag, or equivalent)	Nasco # B01196WA, or equivalent
Swab, cotton	MLS

Item	Source
Reagents	
Media‡:	
Buffered *Listeria* Enrichment Broth (BLEB), 500 g	VWR # EM1.09628.0500
Listeria Selective Enrichment Supplement,§ 16 × 1 mg/vial	VWR # EM1.11781.0001
D/E Neutralizing Broth	BD Catalog # 281910, or equivalent
Half Fraser Broth Base	MLS
Half Fraser Broth Supplement§	MLS
Ottaviani & Agosti agar (OAA, for confirmation tests)	MLS
Nuclease-free Water	Life Technologies Cat. no. AM9938

‡ Only Half Fraser and Fraser broth are part of the NF Validation™ studies (for more details, refer to "NF Validation™ by AFNOR Certification" on page 21)

§ Add supplement to base according to manufacturer's recommendation.

Methods

Workflow

Food samples	Environmental swab samples	Environmental sponge samples
Combine food sample with broth (1:9) ▼ Homogenize the sample ▼ Incubate at 37±1°C for 24–28 hr. ▼	Add swab to 10 mL broth ▼ Incubate at 37±1°C for 28–32 hr. ▼	Add sponge to 100 mL broth ▼ Homogenize the sample ▼ Incubate at 37±1°C for 28–32 hr. ▼

Preparation of DNA—standard protocol (all samples)	Preparation of DNA—extra-clean protocol (food samples)
Filter the enriched sample through the spin column	Filter the enriched sample through the spin column
Load 750 µL of enriched sample onto spin column/tube assembly (*with* zirconium beads). ▼ Spin at 12,000–16,000 × *g* for ~3 min. ▼ Aspirate and discard the supernatant. ▼	Load 750 µL of enriched sample onto spin column/tube assembly (*without* zirconium beads). ▼ Spin at 12,000–16,000 × *g* for ~3 min. ▼ Aspirate and discard the supernatant. ▼
Wash the pellet	Wash the pellet and transfer to the zirconium beads
Resuspend in 500 µL of Nuclease-free Water. (No transfer; zirconium beads already present.) ▼ Spin at 12,000–16,000 × *g* for ~3 min. ▼ Aspirate and discard the supernatant. ▼	Resuspend in 500 µL of Nuclease-free Water. ▼ Transfer to a clean tube with zirconium beads. ▼ Spin at 12,000–16,000 × *g* for ~3 min. ▼ Aspirate and discard the supernatant. ▼

Lyse the sample
Add 50 µL of Lysis Buffer to the pellet, bead-beat for ~5 min., and spin down (~10 sec.). ▼ Incubate at 97±2°C for ~10 min., cool at room temp. for ~2 min., and spin down (~1 min.). ▼ Add 250 µL of Nuclease-free Water and spin at 12,000–16,000 × *g* for ~1 min. (DNA is in supernatant.) ▼
Proceed to PCR or store the DNA sample

Sample enrichment

Procedural guidelines

IMPORTANT! Use proper aseptic technique while handling samples to avoid cross-contamination.

We have validated storage of cultures enriched in Half Fraser Broth at 5±3°C for up to 72 hours prior to DNA preparation. This extended storage period allows for convenient weekend or holiday storage.

Before you begin: prepare enrichment broth

For the sample types below, prepare either:

- BLEB by combining BLEB Base with *Listeria* Selective Enrichment Supplement as directed by the manufacturer.
  or
- Half Fraser broth†, either ready to use, or by combining Fraser Broth Base with Fraser Broth Supplement, as directed by the manufacturer.

Enrichment of food sample

1. Combine the food sample up to 25 g with prepared broth in a 1:9 ratio. When claiming presence/absence in 25-g samples, ensure this sample volume is used.
   For example, add 225 mL of broth to 25 g (or 25 mL) of food sample.
2. Homogenize the food sample in a homogenizer bag as described below.
   A filtered bag may be used for enrichment of particulated samples.

For these food types...	Homogenize by...
• Solid foods, such as chicken wings • Liquids or powdered foods	Hand massage by squeezing the bag 5–10 times.
• Coarse food types, such as meat and seafood • Soft food types, such as mayonnaise	Process for 1 minute in a homogenizer (Stomacher® 400 Laboratory Blender or equivalent).

3. Incubate the food sample at 37±1°C under static conditions for 24–28 hours.
4. Proceed to DNA preparation, standard (page 14) or extra-clean (page 16) protocol.

Enrichment of environmental swab samples

1. Collect and enrich the environmental sample.
   a. Pre-wet the swab with 0.5 mL of D/E Neutralizing Broth.
   b. Wipe the surface area to be tested.
   c. Add the swab to 10 mL of prepared BLEB or Half Fraser broth in a 15-mL conical tube.
2. Twirl the swab for approximately 1 minute.
3. Incubate the swab at 37±1°C under static conditions for a total incubation time of 28–32 hours.
4. Proceed to the standard DNA preparation protocol (page 14).

† Only Half Fraser and Fraser broth are part of the NF Validation™ studies (for more details, refer to "NF Validation™ by AFNOR Certification" on page 21)

Enrichment of environmental sponge samples

1. Collect and enrich the environmental sample.
   a. Pre-wet the sponge with 10 mL of D/E Neutralizing Broth.
   b. Wipe the surface area to be tested.
   c. Add the sponge to 100 mL of BLEB or Half Fraser broth.
2. Homogenize the environmental sample: Use a homogenizer bag (no mesh) and process for 1 minute in a homogenizer (Stomacher® 400 Laboratory Blender or equivalent), or hand squeeze for approximately 1 minute.
3. Incubate the sponge at 37±1°C under static conditions for a total incubation time of 28–32 hours.
4. Proceed to the standard DNA preparation protocol (page 14).

Important procedural guidelines for DNA preparation

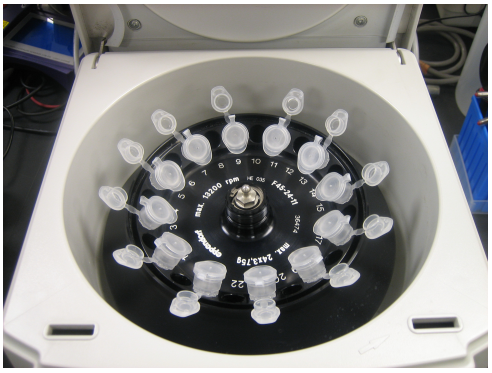
PCR-clean water

Use Nuclease-free Water (Cat. no. AM9938) to wash the bacterial/bead pellets and to dilute the sample after lysis, as it is considered "PCR-clean" water. Autoclaved water should **not** be considered PCR-clean water.

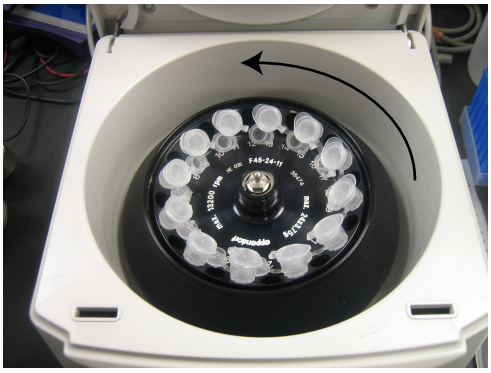
Position of the spin column/tube assembly in the microcentrifuge

- When loading spin column/tube assemblies into the microcentrifuge, place the tube cap hinge toward the inside of the rotor (Figure 1, right), to prevent interference of the cap hinge with installation of the rotor lid.
- To prevent damage to the tube cap during centrifugation, position the cap in the opposite direction of rotation.

Figure 1 Spin column/tube assembly position



Incorrect position of the tube cap



Correct position of the tube cap

Guidelines for samples with high fat content

After filtration through the spin column

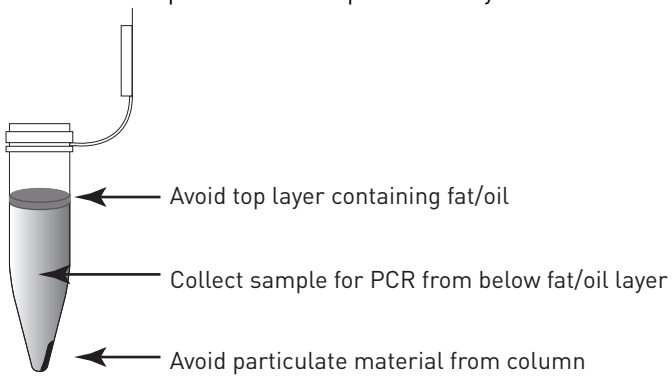
For samples that contain a distinct top fat layer following centrifugation through the spin column, remove the fat layer and supernatant as follows:

Type of fat layer	Fat layer and supernatant removal
Liquid (for example, as found in milk samples)	Use a P1000 pipettor to remove fat from the top surface by aspirating in a circular motion without disturbing the pellet. Continue to collect supernatant from the top surface until all the supernatant is removed. Discard the supernatant into a waste container.
Solid fat layer (for example, as found in infant formula samples)	Use a pipettor to gently dislodge the fat layer without disturbing the pellet. Aspirate the supernatant from the top surface using a pipettor until all the supernatant is removed. Discard the supernatant into a waste container.

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 fatty layer and bottom pellet.

Figure 2 Collect sample from middle phase after lysis



Preparation of DNA—standard protocol

This protocol is designed for the PrepSEQ® Rapid Spin Sample Prep Kit – Bead Beating kit.

Before you begin

- Familiarize yourself with "Important procedural guidelines for DNA preparation" on page 13.
- Set the block heater temperature to 95°C.
- Label zirconium bead-containing 1.5-mL microcentrifuge tubes as needed.

Filter the enriched sample through the spin column

1. Insert a spin column into a labeled microcentrifuge tube that contains zirconium beads.
2. Load 750 µL of the enriched sample onto the spin column, and cap the column.

3. Load the tube with its spin column into the microcentrifuge.

IMPORTANT! Place the tube cap hinge toward the inside of the rotor, and position the cap in the opposite direction of rotation as described in Figure 1 on page 13.

4. Microcentrifuge the tube at 12,000–16,000 × *g* for approximately 3 minutes.
5. Remove the tube from the microcentrifuge, and discard the used spin column.
6. Aspirate and discard the supernatant.

   Remove the supernatant as completely as possible, including any excess liquid on the sides of the tube. Remove droplets by circling the inside of the tube with the pipettor and pushing into supernatant for removal by aspiration.

IMPORTANT! For samples that contain a distinct top fat layer following centrifugation, remove the fat layer as described in "Guidelines for samples with high fat content" on page 14.

Note: The bacterial pellet is a black pellet beneath the zirconium beads.

Wash the pellet

1. Add 500 µL of Nuclease-free Water to the zirconium bead/bacterial pellet, and vortex the sample for about 5 seconds.
2. Microcentrifuge the tube at 12,000–16,000 × *g* for approximately 3 minutes.
3. Carefully aspirate the entire supernatant, removing as few of the zirconium beads as possible, and discard the supernatant.

Lyse the sample

1. Add 50 µL of Lysis Buffer to the zirconium bead/bacterial pellet.
2. Cap the tube, then bead-beat the sample using the Vortex-Genie® mixer at a speed of 8±1 for approximately 5 minutes.

   Note: If you are using the Vortex Adaptor (Cat. no. AM10024) on the mixer, position the tubes with the tube caps facing out. This ensures that the tubes remain seated in the Vortex Adaptor.
3. Microcentrifuge the tube briefly at 12,000–16,000 × *g* for approximately 10 seconds to bring the contents down to the bottom of the tube.
4. Incubate at 97±2°C for approximately 10 minutes to lyse the sample.
5. Allow the sample to cool at room temperature (23±5°C) for approximately 2 minutes.
6. Microcentrifuge the tube at 12,000–16,000 × *g* for approximately 1 minute to bring down condensation.
7. Add 250 µL of Nuclease-free Water, and mix thoroughly.
8. Microcentrifuge the tube at 12,000–16,000 × *g* for approximately 1 minute to bring down any particulate material derived from the spin column, which can interfere with amplification. The microbial DNA is in the aqueous supernatant (~300 µL).

9. Proceed in one of the following ways.
   - Proceed directly to PCR with one of the following kits, using 30 µL of supernatant for each assay.
     – MicroSEQ® *Listeria monocytogenes* Detection Kit (Cat. no. 4403874)
     – MicroSEQ® *Listeria* spp. Detection Kit (Cat. no. 4427410).

     Note: If a fat/debris layer is present on top of the supernatant, collect the DNA sample from the clear middle phase, avoiding the fat layer and the bottom pellet, as described in Figure 2 on page 14.

     (Optional) Remove the remaining supernatant to a new microcentrifuge tube, and store the sample as described in"DNA sample storage before real-time PCR" on page 18 for retesting, if needed.
   - If storage of the DNA sample before PCR is desired, remove the aqueous supernatant (or the clear middle phase if a top fat/debris layer is present) to a new microcentrifuge tube, and store the sample as described in"DNA sample storage before real-time PCR" on page 18.

Preparation of DNA—extra-clean protocol

This protocol is designed for the PrepSEQ® Rapid Spin Sample Prep Kit – Extra Clean & Bead Beating.

Before you begin

- Familiarize yourself with "Important procedural guidelines for DNA preparation" on page 13.
- Set the block heater temperature to 95°C.
- Label 1.5-mL microcentrifuge tubes, with and without zirconium beds, as needed.

Filter the enriched sample through the spin column

1. Insert a spin column into an empty labeled microcentrifuge tube.

   Note: Do not use the tube containing zirconium beads.
2. Load 750 µL of the enriched sample onto the spin column and cap the column.
3. Load the tube with its spin column into the microcentrifuge.

   IMPORTANT! Place the tube cap hinge toward the inside of the rotor, and position the cap in the opposite direction of rotation (Figure 1 on page 13).
4. Microcentrifuge the tube at 12,000–16,000 × *g* for approximately 3 minutes.
5. Remove the tube from the microcentrifuge, then discard the used spin column.

6. Aspirate and discard the supernatant.

   Remove the supernatant as completely as possible, including any excess liquid on the sides of the tube. Remove droplets by circling the inside of the tube with the pipettor and pushing into supernatant for removal by aspiration.

   IMPORTANT! For samples that contain a distinct top fat layer following centrifugation, remove the fat layer as described in "Guidelines for samples with high fat content" on page 14.

   Note: The bacterial pellet is a black pellet beneath the zirconium beads.

Wash the pellet and transfer to the zirconium beads

1. Add 500 µL of Nuclease-free Water to the bacterial pellet, and resuspend the pellet by pipetting up and down.
2. Resuspend the pellet by pipetting up and down.
3. Transfer the sample to a clean microcentrifuge tube containing zirconium beads.

   IMPORTANT! Avoid contact with residual fat on the sides of the tube. Transfer only the Nuclease-free Water containing the resuspended pellet.

4. Microcentrifuge the tube at 12,000–16,000 × *g* for approximately 3 minutes.
5. Carefully aspirate the supernatant at the position opposite the sample pellet, and discard the supernatant.

Lyse the sample

1. Add 50 µL of Lysis Buffer to the tube.
2. Cap the tube, then bead-beat the sample using the Vortex-Genie® mixer at a speed of 8±1 for approximately 5 minutes.

   Note: If you are using the Vortex Adaptor (Cat. no. AM10024) on the mixer, position the tubes with the tube caps facing out. This ensures that the tubes remain seated in the Vortex Adaptor.

3. Microcentrifuge the tube briefly at 12,000–16,000 × *g* for approximately 10 seconds to bring the contents down to the bottom of the tube.
4. Incubate at 97±2°C for approximately 10 minutes to lyse samples.
5. Allow the sample to cool at room temperature (23±5°C) for approximately 2 minutes.
6. Microcentrifuge the tube at 12,000–16,000 × *g* for approximately 1 minute to bring down condensation.
7. Add 250 µL of Nuclease-free Water, and mix thoroughly.
8. Microcentrifuge the tube at 12,000–16,000 × *g* for approximately 1 minute to bring down any particulate material derived from the spin column, which can interfere with amplification. The microbial DNA is in the aqueous supernatant.

9. Proceed in one of the following ways.
   - Proceed directly to PCR with one of the following kits, using 30 µL of supernatant for each assay.
     – MicroSEQ® *Listeria monocytogenes* Detection Kit (Cat. no. 4403874)
     – MicroSEQ® *Listeria* spp. Detection Kit (Cat. no. 4427410).

     Note: If a fat/debris layer is present on top of the supernatant, collect the DNA sample from the clear middle phase, avoiding the fat layer and the bottom pellet, as described in Figure 2 on page 14.

     (Optional) Remove the remaining supernatant to a new microcentrifuge tube, and store the sample as described in"DNA sample storage before real-time PCR" on page 18 for retesting, if needed.
   - If storage of the DNA sample before PCR is desired, remove the aqueous supernatant (or the clear middle phase if a top fat/debris layer is present) to a new microcentrifuge tube, and store the sample as described in"DNA sample storage before real-time PCR" in the next section.

DNA sample storage before real-time PCR

1. After removal of the aqueous supernatant containing the DNA to a new microcentrifuge tube, store the DNA samples in one of the following ways:
   - At 5±3°C for up to 72 hours
   - Below –18°C for long-term storage
2. Centrifuge the stored DNA sample at 12,000–16,000 × *g* for approximately 1 minute to pellet remaining debris and remove condensation prior to opening the tube.

Troubleshooting

Observation	Possible cause	Recommended action
Inhibition of PCR, indicated by: • Warning calls with the RapidFinder™ Express software • Non-detection of the internal positive control with SDS or StepOne® software	Removal of the supernatant before adding Lysis Buffer was not sufficient.	Dilute 5 µL of DNA with 25 µL of water and repeat the PCR with the diluted sample.
	Filtrate from the spin column is in the PCR.	Centrifuge the sample to separate the filter particulates before adding sample to the PCR reaction. Pipette your sample from the clear center section, avoiding the particulate material at the bottom of the tube.
	The sample contained excess fat that was not removed during aspiration of the supernatant.	Apply PrepSEQ® Rapid Spin Bead-Beating Extra-Clean Sample Prep protocol when processing a matrix with high fat content.
The bacterial pellet separates from the tube, making the pellet difficult to avoid during aspiration	Sample was left unattended before the supernatant was aspirated.	Centrifuge again and remove supernatant immediately.

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NF Validation™ by AFNOR Certification

Visit **www.lifetechnologies.com/foodsafety** for a complete list of certified workflows for detection of *L. mono* and *L.* spp.

Certification	Expiration
 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** or **www.lifetechnologies.com**‡.

‡ In the Product Literature section of the product web page.

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. NF Validation™ by AFNOR Certification; **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 24–48 hours 2. 37±1°C for 16–24 hours
PrepSEQ® Rapid Spin Sample Prep Kits (Bead Beating)	Half Fraser Broth	37±1°C for 24–48 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 (see Pub. no. 4470047)
  - PrepSEQ® Rapid Spin Sample Prep Kit – Bead Beating (this user guide)
  - PrepSEQ® Rapid Spin Sample Prep Kit – Extra Clean and Bead Beating (for meat samples, this user guide)
- *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 23

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 the NF Validation™ workflow: 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.

B

Safety

CAUTION! 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

CAUTION! 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.

Biological hazard safety

CAUTION! 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**

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.

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