USER GUIDE

applied biosystems by life technologies™

PrepSEQ® Rapid Spin Sample Preparation Kit: *Listeria* spp.

Preparation of MicroSEQ® PCR-ready DNA from food and environmental samples

Catalog Numbers 4426714, 4426715

Publication Number 4426506
Revision C

For testing of Food and Environmental samples only.

life technologies™

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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
C	November 2013	• Added certification details for AOAC® Performance Tested Methods℠ certification. • Updated number format (time, temperature, and centrifugation speeds) for AOAC® certification. • Updated to a Life Technologies template with associated updates to the limited license information, warranty information, trademark statement, and safety statements.
B	June 2010	Baseline for revision history.

1 Product information

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

Overview

Use the PrepSEQ® Rapid Spin Sample Preparation Kit to prepare food and environmental samples to test for *Listeria* spp. The kit procedure involves:

- Enrichment of food and environmental samples for *Listeria* spp.
- Sample preparation

For sample preparation from enriched food and environmental samples, we recommend a 750-µL sample volume.

For some foods with a high lipid content, such as infant formula, whole milk, smoked salmon (lox), and mayonnaise, use the PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K (Cat. no. 4426715).

See Appendix A, "Supplemental information" for detailed information about AOAC® certification.

The PrepSEQ® Rapid Spin Sample Preparation Kit is for professional use only. Users may include, but are not limited to, food producers, food processors, food manufacturers, retailers, and microbiology testing laboratories.

Visit **www.lifetechnologies.com/foodsafety** for a list of workflows for detection of *Listeria* spp.

Kit contents

The PrepSEQ® Rapid Spin Sample Preparation Kits contains reagents for 100 sample preparations.

Item	Quantity or volume	Storage
PrepSEQ® Rapid Spin Sample Preparation Kit with Proteinase K (Cat. no. 4426714)		
Spin columns	100	Room temperature (23±5°C)
Microcentrifuge tubes, 1.5 mL	100	Room temperature (23±5°C)
Lysis Buffer, 1 bottle	5 mL	5±3°C
Proteinase K (20 mg/mL), 1 tube	1.25 mL	Below –18°C
PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K (Cat. no. 4426715)		
Spin columns	100	Room temperature (23±5°C)
Microcentrifuge tubes, 1.5 mL	2 × 100	Room temperature (23±5°C)
Lysis Buffer, 1 bottle	5 mL	5±3°C
Proteinase K (20 mg/mL), 1 tube	1.25 mL	Below –18°C

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

Materials not included in the kit

The materials listed here have been validated for use with this kit. Results may vary if substituted products from other vendors are used instead. Unless otherwise indicated, all materials are available from Life Technologies. MLS: Major laboratory supplier.

Item	Source
Equipment	
Block heater, 56°C	MLS
Block heater, 95°C	MLS
Rack for 1.5-mL tubes	MLS
Benchtop microcentrifuge	Eppendorf 5415 D or equivalent

Item	Source
Homogenizer (Stomacher® 400 Laboratory Blender or equivalent)	Seward # 0400/001/AJ or equivalent
Vortexer	MLS
Consumables	
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS
Pipettors: • Positive-displacement • Air-displacement	MLS
Swab, cotton	MLS
15-mL conical tubes	MLS
Homogenizer bags appropriate for your sample:	
Enrichment Bag with sponge, 4.5" × 9", (Whirl-Pak® Speci-Sponge Bags or equivalent)	Nasco Catalog # B01245WA or equivalent
Enrichment Bag with mesh, 6" × 9", 24 oz, (Whirl-Pak® Filter Bags or equivalent)	Nasco Catalog # B01348WA or equivalent
Enrichment Bag, no mesh, 6" × 9", 24 oz, (Whirl-Pak® Standard Bags or equivalent)	Nasco Catalog # B01196WA or equivalent
Reagents	
Dey Engley (D/E) Neutralizing Broth	BD Catalog # 281910 or equivalent
Buffered *Listeria* Enrichment Broth (BLEB), 500 g	VWR # EM1.09628.0500
Listeria Selective Enrichment Supplement, 16 × 1 mg/vial	VWR # EM1.11781.0001
Nuclease-free Water	Cat. no. AM9938

2 PrepSEQ® Rapid Spin Sample Preparation Kit: *Listeria* spp.

Before you begin

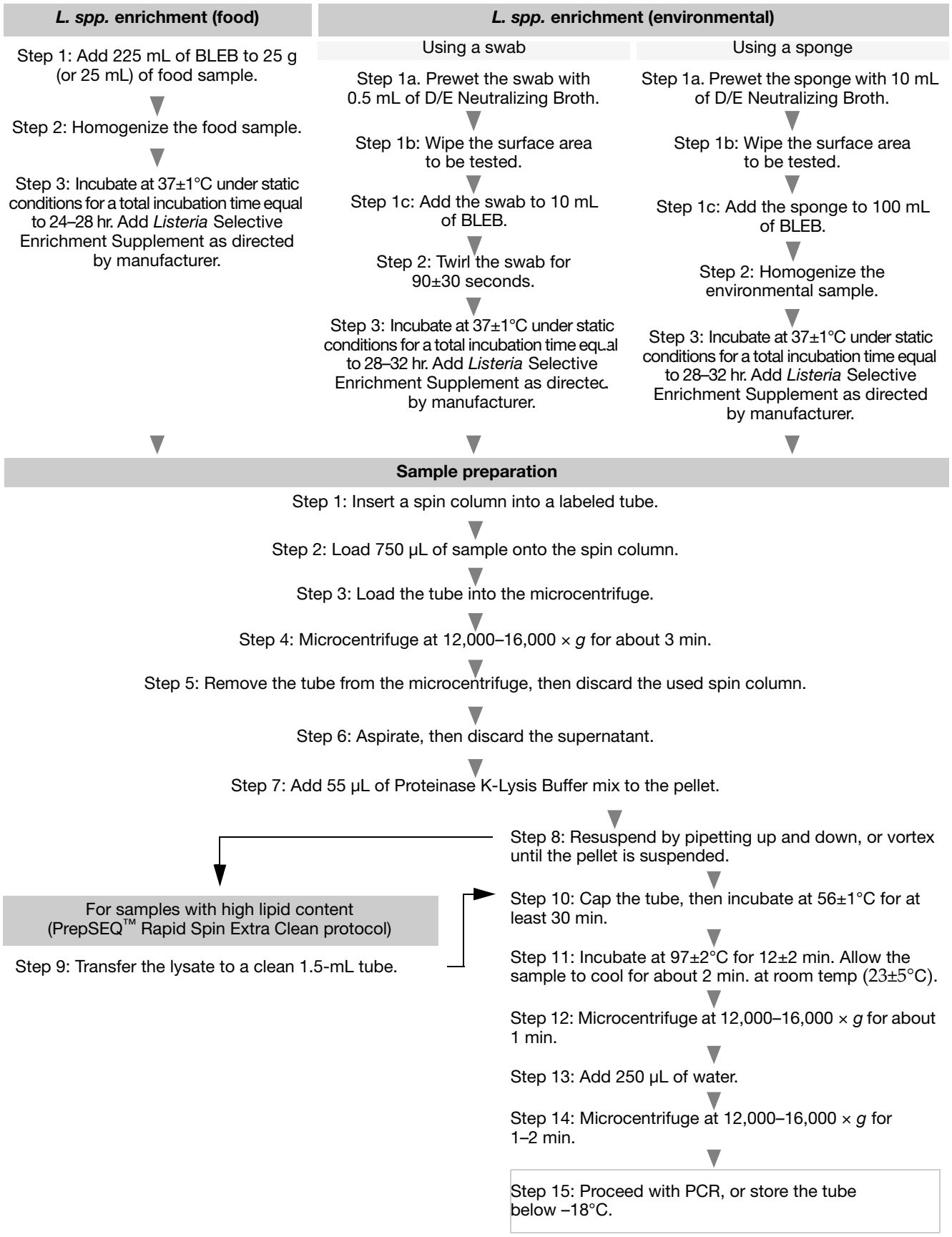
Before starting your sample extraction:

- Set one block heater temperature to 95°C, and the other block heater temperature to 56°C.
- Label 1.5-mL microcentrifuge tubes.
- For processing multiple samples, we recommend preparing a Proteinase K-Lysis Buffer mix:

  Premix 5 µL of Proteinase K (20 mg/mL) with 50 µL of Lysis Buffer for each sample (use a clean appropriately-sized container for mixing). Multiply volumes by the number of samples plus 10% for overage. Mix well to disperse Proteinase K in Lysis Buffer. Use immediately, or store on ice until ready to use.

Kit workflow

The following is a sample preparation workflow for the procedures starting on page 13.


L. spp. enrichment (food)
Step 1: Add 225 mL of BLEB to 25 g (or 25 mL) of food sample.
Step 2: Homogenize the food sample.
Step 3: Incubate at 37±1°C under static conditions for a total incubation time equal to 24–28 hr. Add *Listeria* Selective Enrichment Supplement as directed by manufacturer.
L. spp. enrichment (environmental)
Using a swab
Step 1a. Prewet the swab with 0.5 mL of D/E Neutralizing Broth.
Step 1b: Wipe the surface area to be tested.
Step 1c: Add the swab to 10 mL of BLEB.
Step 2: Twirl the swab for 90±30 seconds.
Step 3: Incubate at 37±1°C under static conditions for a total incubation time equal to 28–32 hr. Add *Listeria* Selective Enrichment Supplement as directed by manufacturer.
Using a sponge
Step 1a. Prewet the sponge with 10 mL of D/E Neutralizing Broth.
Step 1b: Wipe the surface area to be tested.
Step 1c: Add the sponge to 100 mL of BLEB.
Step 2: Homogenize the environmental sample.
Step 3: Incubate at 37±1°C under static conditions for a total incubation time equal to 28–32 hr. Add *Listeria* Selective Enrichment Supplement as directed by manufacturer.
Sample preparation
Step 1: Insert a spin column into a labeled tube.
Step 2: Load 750 µL of sample onto the spin column.
Step 3: Load the tube into the microcentrifuge.
Step 4: Microcentrifuge at 12,000–16,000 × *g* for about 3 min.
Step 5: Remove the tube from the microcentrifuge, then discard the used spin column.
Step 6: Aspirate, then discard the supernatant.
Step 7: Add 55 µL of Proteinase K-Lysis Buffer mix to the pellet.
Step 8: Resuspend by pipetting up and down, or vortex until the pellet is suspended.
For samples with high lipid content (PrepSEQ™ Rapid Spin Extra Clean protocol)
Step 9: Transfer the lysate to a clean 1.5-mL tube.
Step 10: Cap the tube, then incubate at 56±1°C for at least 30 min.
Step 11: Incubate at 97±2°C for 12±2 min. Allow the sample to cool for about 2 min. at room temp (23±5°C).
Step 12: Microcentrifuge at 12,000–16,000 × *g* for about 1 min.
Step 13: Add 250 µL of water.
Step 14: Microcentrifuge at 12,000–16,000 × *g* for 1–2 min.
Step 15: Proceed with PCR, or store the tube below –18°C.

Sample enrichment

Enrich food sample

1. Add 225 mL of Buffered *Listeria* Enrichment Broth (BLEB) to 25 g (or 25 mL) of food sample.

   Note: This protocol was validated using Buffered *Listeria* Enrichment Broth (VWR # EM1.09628.0500). Other sources of media might provide different results.

2. Homogenize the food sample:
   - For coarse food types, such as meat, poultry, and seafood, use a filtered stomacher bag and homogenize for about 1 minute (Stomacher® 400 Laboratory Blender speed setting **Norm**, or equivalent).
   - For soft food types, such as mayonnaise or soft cheese, use a nonfiltered stomacher bag and homogenize for about 1 minute (Stomacher® 400 Laboratory Blender speed setting **Norm**, or equivalent).
   - For liquids or powdered foods, use a nonfiltered Stomacher® bag and hand massage by squeezing the bag 5–10 times.
3. Incubate at 37±1°C under static conditions for a total incubation time equal to 24–28 hours.
   a. After 4±0.25 hours of incubation or as directed by manufacturer, add *Listeria* Selective Enrichment Supplement.
4. Proceed to "Preparation and extraction of DNA" on page 14.

Enrich environmental sample using a swab

1. Collect and enrich the environmental sample.
   a. Prewet 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 BLEB in a 15-mL conical tube.
2. Twirl the swab for 90±30 seconds.
3. Incubate at 37±1°C under static conditions for a total incubation time equal to 28–32 hours.
   a. After 4±0.25 hours of incubation or as directed by manufacturer, add *Listeria* Selective Enrichment Supplement.
4. Proceed to "Preparation and extraction of DNA" on page 14.

Enrich environmental sample using a sponge

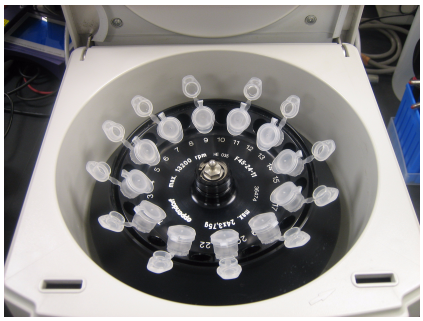
1. Collect and enrich the environmental sample.
   a. Prewet the sponge with 10 mL of D/E Neutralizing Broth.
   b. Wipe the surface area to be tested.
   c. Add the sponge to 100 mL of BLEB.
2. Homogenize the environmental sample: Use a nonfiltered nonmesh stomacher bag and homogenize for about 1 minute (Stomacher® 400 Laboratory Blender speed setting **Norm**, or equivalent), or hand squeeze for about 1 minute.
3. Incubate at 37±1°C under static conditions for a total incubation time equal to 28–32 hours.
   a. After 4±0.25 hours of incubation or as directed by manufacturer, add *Listeria* Selective Enrichment Supplement.
4. Proceed to "Preparation and extraction of DNA".

Preparation and extraction of DNA

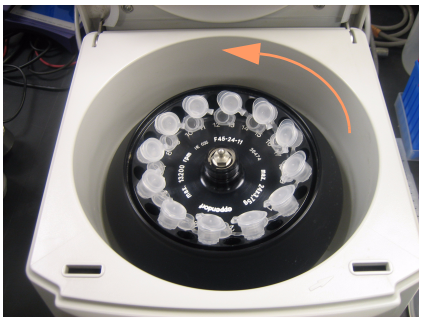
1. Insert a spin column into a labeled tube.
2. Load 750 µL of your enriched sample onto the spin column and cap the column.

   Note: For environmental samples, squeeze the sponges or twirl the swab 2–3 times before sampling.
3. Load the tube with its spin column into the microcentrifuge. Place the tube cap hinge toward the inside of the rotor (see figure at right below), otherwise the cap hinge interferes with installation of the rotor lid.

   Note: To prevent damage to the tube cap during centrifugation, position the cap in the opposite direction of rotation.



Incorrect position of the tube cap



Correct position of the tube cap

4. Microcentrifuge the tube at 12,000 to 16,000 × g for about 3 minutes.
5. Remove the tube from the microcentrifuge, then discard the used spin column.

6. Aspirate, then discard the supernatant.

IMPORTANT! Remove 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 fat layer following centrifugation, indicated as a distinct top layer, remove the fat layer as follows:

- For liquid fat layer (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.

or

- For solid fat layer (for example, as found in infant formula samples): Use a pipettor to gently dislodge the fat layer without disturbing the pellet. Pour off the supernatant and fat layer using a single quick motion. Aspirate the supernatant from the top surface using a pipettor until all the supernatant is removed. Discard the supernatant into a waste container.

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7. Add 55 µL of Proteinase K-Lysis Buffer mix (see page 11) to the pellet.

IMPORTANT! Store Proteinase K-Lysis Buffer mix on ice until ready to use.

Note: Proteinase K is required for efficient lysis of *Listeria monocytogenes.*

8. Resuspend by pipetting up and down, or vortex until the pellet is well dispersed in the Proteinase K-Lysis Buffer mix.

9. **Rapid Spin Extra Clean protocol *only*** – If the food sample has high lipid content, transfer the mixture into a clean 1.5-mL tube (avoid transferring the fat when transferring the mixture). The pellet must be well dispersed in the Proteinase K-Lysis Buffer prior to transfer. For all other samples, proceed directly to step 10.

IMPORTANT! During the transfer there will be residual fat on the sides of the original tube. Avoid contact with the fat and transfer only the Lysis Buffer containing the resuspended pellet into a clean tube.

IMPORTANT! We recommend the Rapid Spin Extra Clean protocol step for food samples with high lipid content, such as found with infant formula, whole milk, smoked salmon (lox), and mayonnaise; for use with PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean (Cat. no. 4426715).

Note: Inhibition of PCR with the detection assay will be indicated by warning calls with the RapidFinder™ Express software or non-detection of the internal positive control with SDS or StepOne™ software. If your matrix has high fat content and if inhibition of PCR is frequently observed, we recommend applying the Rapid Spin Extra Clean protocol step to the matrix.

10. Cap the tube, then incubate at 56±1°C for at least 30 minutes to activate the Proteinase K.

11. Incubate at 97±2°C for 12±2 minutes to lyse samples and inactivate Proteinase K, then allow the sample to cool for about 2 minutes at room temperature (23±5°C).
12. Microcentrifuge the tube at 12,000–16,000 × *g* for about 1 minute to bring down condensation following the 95°C heating step.
13. Add 250 µL of water. Mix well.

IMPORTANT! Use PCR-clean water (Cat. no. AM9938). Autoclaved water should not be considered PCR-clean.

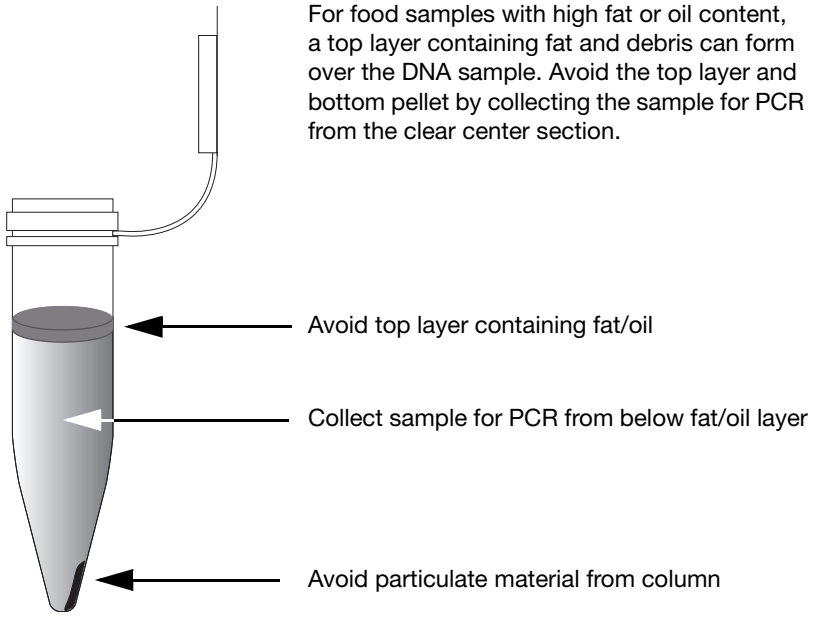
14. Microcentrifuge the tube at 12,000–16,000 × *g* for 1–2 minutes to bring down any particulate material derived from the spin column, which can interfere with amplification. The microbial DNA is in the aqueous phase.
15. Proceed with PCR, or store the tube below –18°C.

IMPORTANT! For PCR, add 30 µL of supernatant to the lyophilized assay. For detailed instructions, refer to the MicroSEQ® *Listeria* spp. Detection Kit User Guide (Pub. no. 4426510).

IMPORTANT! Food samples with high fat or oil content can form a top layer containing fat and debris over the DNA sample. When collecting your sample for PCR, avoid the top layer and bottom pellet by collecting your sample from the clear center section (see figure below).

IMPORTANT! If PCR inhibition occurs, as indicated by no amplification of both target and IPC, then dilute sample with water. We recommend adding 5 µL of sample and 25 µL of water to the lyophilized assay to overcome inhibition. See "Troubleshooting" on page 17 for further discussion.

To pipette eluate from foods with high fat or oil content:



Troubleshooting

Observation	Possible cause	Action
Inhibition of PCR, indicated by non-detection of IPC reaction	Removal of the supernatant before adding Lysis Buffer was not sufficient.	Add 5 µL of sample and 25 µL of water to the lyophilized assay to overcome inhibition.
	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 Extra Clean protocol when processing a matrix with high fat content.
	The matrix is associated with PCR inhibitory components.	Pre-wash the bacterial pellet before column clarification: **1.** Transfer 750 µL of sample to a clean microcentrifuge tube. **2.** Centrifuge at 12,000–16,000 × *g* for about 3 min. **3.** Discard supernatant. **4.** Resuspend pellet in 650 µL of sterile distilled water. **5.** Load column and centrifuge. Continue with sample prep step 3 on page 14.
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.

A Supplemental information

Background information and 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 several recently discovered new species, given the provisional names *L. marthii*, *L. rocourti*, *L. fleischmannii*, and *L. weihenstephanensis*. *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.

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

AOAC® *Performance Tested Methods*℠ Certification

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

Workflow

The PrepSEQ® kits and the MicroSEQ® *Listeria* spp. Detection Kit 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
- MicroSEQ® *Listeria* spp. Detection Kit
- 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:

Reference method	Matrix
ISO 11290–1:1996 with Amendment 1:2004	**Foods**: pasteurized whole cow's milk, dry infant formula, hot dogs, roast beef, lox **Environmental surfaces**: stainless steel, plastic cutting board, ceramic tile, rubber sheets and 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.

B

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.

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**

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

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20 November 2013