USER GUIDE

applied biosystems by life technologies™

# PrepSEQ® Rapid Spin Sample Preparation Kits

Isolation of PCR-ready DNA from *Campylobacter jejuni/coli/lari* in food samples

For use with:

PrepSEQ® Rapid Spin Sample Preparation Kit
PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean

**Catalog Numbers** 4407760, 4413269

**Publication Number** 4466620
**Revision** B

**For testing of Food and Environmental samples only.**

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# About this guide

## Revision history

| Revision | Date | Description |
|---|---|---|
| B | April 2014 | • Updated references to the downstream kit for real-time PCR, whose name changed from *MicroSEQ® Campylobacter jejuni/coli/lari Detection Kit* (custom assay) to *RapidFinder™ Campylobacter Multiplex Assay Beads* (catalog assay).<br>• Updated recommended enrichment method section for general use.<br>• Added additional troubleshooting tips.<br>• Updated number formatting for temperature, time, and centrifugal speeds.<br>• Updated document template with associated updates to the covers, limited license and warranty information, and trademark and safety statements.<br>• Updated company name from Life Technologies to Thermo Fisher Scientific. |
| A | May 2011 | New user guide. |

# 1 Product information

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

## Product description

### Overview

The PrepSEQ® Rapid Spin Sample Preparation Kits are designed for extraction of PCR-ready microbial DNA from food samples. The kits contain reagents necessary for removal of PCR inhibitors and lysis of microbial organisms. Downstream applications include use in real-time PCR (for example, RapidFinder™ Campylobacter Multiplex Assay Beads, Cat. no. 4485027) to detect food pathogens.

### Sample types and inputs

The PrepSEQ® Rapid Spin Sample Preparation Kit and PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean are designed for preparation of DNA from most food types. For sample preparation from enriched food samples or poultry carcass rinses for *Campylobacter jejuni/coli/lari* testing, we recommend a 750-µL sample volume.

This user guide contains sample preparation procedures that allow you to detect 1–3 colony-forming units in 25 grams of food or 25 mL of enriched poultry carcass rinses, or ≥1000 colony-forming units per mL of direct poultry carcass rinse.

We recommend the PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean:

- For food samples with high lipid content, such as found with infant formula, whole milk, smoked salmon (lox), and chicken wing samples
- If your matrix has high fat content, and inhibition of PCR is frequently observed in the detection assay results (as indicated by non-detection of the internal positive control in the SDS software)

## Kit contents

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

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

| Item | Quantity or volume | Storage |
|---|---|---|
| **PrepSEQ® Rapid Spin Sample Preparation Kit** (Cat. no. 4407760) | | |
| 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 |
| **PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean** (Cat. no. 4413269) | | |
| 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 |

## Required materials not included in the kit

The following table includes materials and equipment required but not included in the kits. Unless otherwise indicated, all materials are available from Life Technologies. MLS: major laboratory supplier.

| Item | Source |
|---|---|
| **Equipment** | |
| Benchtop microcentrifuge | Eppendorf 5415 D or equivalent |
| Block heater, 95°C | MLS |
| Rack for 1.5-mL tubes | MLS |
| Vortexer | MLS |
| **Consumables** | |
| Disposable gloves | MLS |
| Micropipette tips, aerosol-resistant | MLS |
| Pipettors:<br>• Positive-displacement<br>• Air-displacement | MLS |
| **Reagents** | |
| Nuclease-free water | Cat. no. AM9938 |

# Additional materials for enrichment

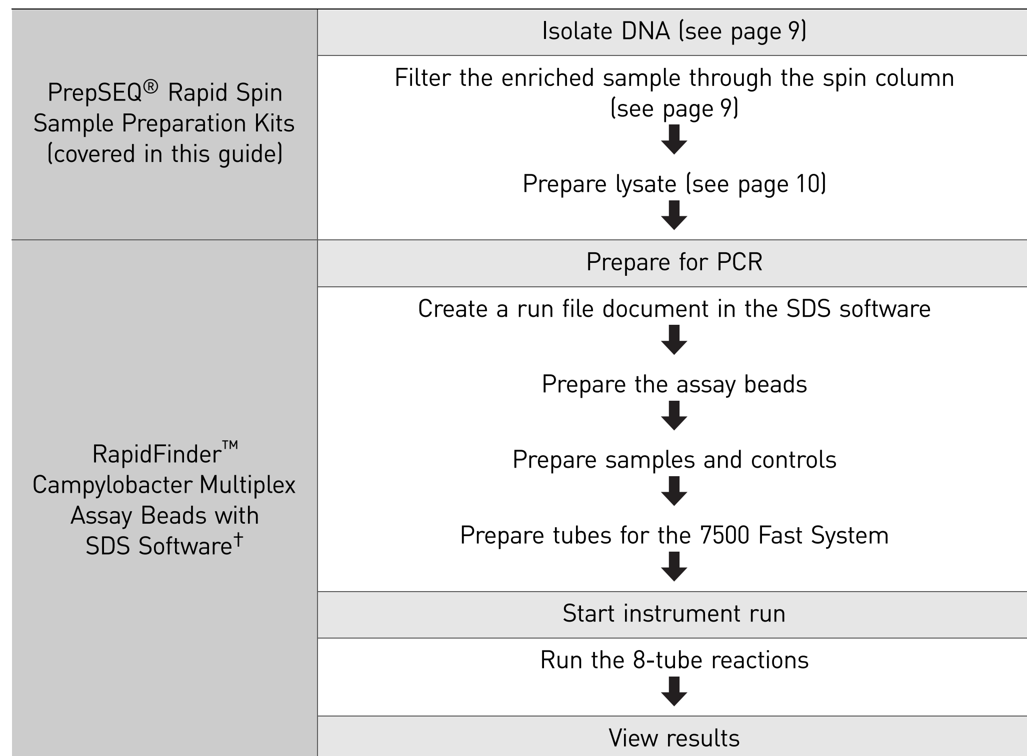
## General recommendations

You will need enrichment reagents and a protocol appropriate for the matrix and pathogen of interest. We recommend that you validate your matrices using a current reference method (for example, ISO 10272-1).

For most food types, the enrichment time for use with a PCR-based pathogen detection kit (for example the RapidFinder™ Campylobacter Multiplex Assay Beads) can be reduced significantly from standard microbiology enrichment protocols. We recommend that you optimize the time needed to enrich your specific sample.

# Workflow

This guide provides instructions for using the PrepSEQ® Rapid Spin Sample Preparation Kits to prepare food samples and poultry carcass rinses specifically for *Campylobacter jejuni/coli/lari* testing. The resulting prepared DNA is compatible for use with the RapidFinder™ Campylobacter Multiplex Assay Beads (Cat. no. 4485027).




† Refer to RapidFinder™ Campylobacter Multiplex Assay Beads *User Guide* (Pub. no. 4466312) for instructions for preparing and running the detection assay.

# 2 Isolate PCR-ready DNA

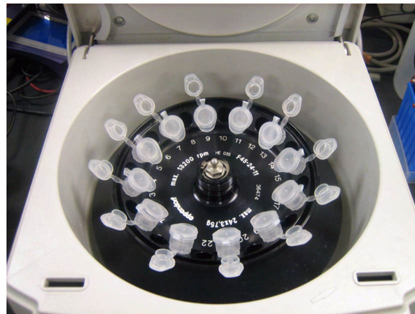
## Before you begin

Before you begin the protocol:

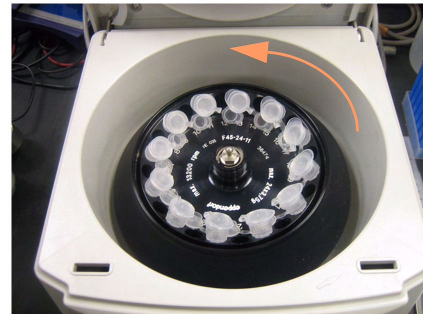
- Set the block heater temperature to 95°C.
- Label the 1.5-mL microcentrifuge tubes.

## Filter the enriched sample through the spin column

1. Insert a spin column into a labeled microcentrifuge tube.
2. Load 750 µL of the enriched sample into the spin column and cap the column.
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). Otherwise the cap hinge interferes with installation of the rotor lid.



Incorrect position of the tube cap



Correct position of the tube cap

4. Microcentrifuge the tube at 12,000–16,000 × *g* for about 3 minutes.
5. Remove the tube from the microcentrifuge and 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 the 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 pipettor to remove fat from the top surface by aspirating in a circular motion. Continue to collect supernatant from the top surface until all the supernatant is removed (discard 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 and pour off the supernatant and fat layer using a single quick motion (discard into a waste container). Remove the remaining supernatant using a pipettor.

## Prepare lysate

1. Add 50 µL of Lysis Buffer to the pellet.
2. Resuspend by pipetting up and down until the pellet is well dispersed.
3. Rapid Spin Extra Clean protocol *only* – If the food sample has high lipid content, transfer the Lysis Buffer mixture into a clean 1.5-mL tube (avoid fat). The pellet must be well dispersed in the Lysis Buffer prior to transfer. For all other samples, proceed directly to step 4.

   IMPORTANT! The lysis buffer mix needs to be transferred to a clean tube. 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.

4. Cap the tube, then incubate at 97±2°C for 12±2 minutes.
5. Allow the sample to cool for about 2 minutes at room temperature (23±5°C).
6. Microcentrifuge the tube at 12,000–16,000 × *g* for about 1 minute to bring down condensation following the 97±2°C heating step.
7. Add 250 µL of water. Mix well.

   IMPORTANT! Use nuclease-free water that is compatible with PCR. Autoclaved water should not be considered compatible with PCR.

8. 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.

   Note: Samples can be stored at 5±3°C overnight, or below –18°C for long term storage. Prior to running PCR, stored samples should be centrifuged at 12,000–16,000 × *g* for 1–2 minutes to pellet the column particulate material.

9. Proceed with PCR, or store the tube below –18°C.

   Use 30 µL of supernatant for each PCR. Refer to the *RapidFinder™ Campylobacter Multiplex Assay Beads User Guide* (Pub. no. 4466312).

# 3 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. | Dilute the sample 1:5 or 1:10 with water to dilute PCR inhibitors. If PCR remains inhibited, repeat the sample preparation. |
| | Filtrate from the spin column is in the sample. | Centrifuge the sample to separate the filter particulates before adding sample to the PCR reaction. |
| | The sample contained excess fat that was not removed during aspiration of the supernatant. | Apply PrepSEQ® Rapid Spin extra clean protocol. |
| | Matrix associated with PCR inhibitory components. | Pre-wash the bacterial pellet before column clarification:<br>1. Transfer 750 µL of sample to a clean microcentrifuge tube.<br>2. Centrifuge at 12,000–16,000 × *g* for about 3 min.<br>3. Discard supernatant.<br>4. Resuspend pellet in 650 µL of sterile distilled water.<br>5. Load column. |
| The bacterial pellet separates from the tube making pellet hard to avoid during aspiration | Sample was left unattended before the supernatant was aspirated. | Remove supernatant immediately following centrifugation. |

# A Supplemental information

## Description of target microorganisms

*Campylobacter jejuni/coli/lari* are major food-borne pathogens that cause diarrhea, periodontitis, and dysentery syndrome in humans. *Campylobacter jejuni* is the leading cause of bacterial diarrheal illness in the United States. It causes more disease than *Salmonella* spp. Outbreaks of campylobacteriosis have been associated with contaminated food supplies such as chicken (20–100% of retail chickens are contaminated), raw milk, and leafy greens.

## Kit sensitivity

The limit of detection using this kit is $10^3$ colony-forming units (cfu)/mL for enriched food matrices, or enriched or direct poultry carcass rinses.

The sensitivity of the assay in real culture samples depends on the quality of the sample preparation method that is used. This user guide contains sample preparation procedures that allow you to detect 1–3 colony-forming units in 25 grams of food or 25 mL of enriched poultry carcass rinses, or ≥1000 cfu/mL of direct poultry carcass rinse.

## Audience

This document is intended for investigators who need to test for *Campylobacter jejuni/coli/lari* in food samples, or enriched or direct poultry carcass rinses.

# 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 an 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 SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

## Biological hazard safety

**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. 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**

# Reference

ISO. 2006. Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of Campylobacter spp. -- Part 1: Detection method. Reference number: ISO 10272-1:2006.

# Documentation and support

## Related documentation

Portable document format (PDF) versions of this guide and the following related documents are available online at **www.lifetechnologies.com**:

| Document | Publication number | Description |
|---|---|---|
| *TaqMan® Campylobacter Multiplex Assay Beads User Guide* | 4466312 | Describes procedures for setting up the SDS software, running PCR, and analyzing results for *Campylobacter jejuni/coli/lari* detection. |

Note: To open the user documentation available online, use the Adobe® Reader® software available from **www.adobe.com**

Note: For additional documentation, see "Obtaining support" on page 18.

## 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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