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PrepSEQ® Nucleic Acid Extraction Kit for Food Testing: *E. coli* O157:H7

Catalog Numbers 4480466, 4428176 **Publication Number** 4426515 Rev. B

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *PrepSEQ® Nucleic Acid Extraction Kit for Food Testing: E. coli O157:H7* (Pub. no. 4426513). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product information

The PrepSEQ® Nucleic Acid Extraction Kit for Food Testing enables preparation of PCR-ready microbial DNA from broth cultures using magnetic-bead based technology.

Refer to the *MicroSEQ® E. coli O157:H7 Detection Kit User Guide* (Pub. no. 4426511) for information about NF Validation™-certified (AFNOR) and AOAC® Performance Tested Methods℠-certified workflows for *E. coli* O157:H7. Visit **www.lifetechnologies.com/foodsafety** for a comprehensive list of workflows for detection of *E. coli.*

Kit contents and storage

Item	Cat. no. 4480466 (100 reactions)	Cat. no. 4428176 (300 reactions)	Storage
Lysis Buffer	2 × 50 mL	6 × 50 mL	Room temperature (23±5°C)
Magnetic Particles	2 × 1.5 mL	6 × 1.5 mL	5±3°C
Binding Solution (Isopropanol)	1 empty bottle	3 empty bottles	Room temperature (23±5°C)
Wash Buffer Concentrate	2 × 26 mL	6 × 26 mL	Room temperature (23±5°C)
Elution Buffer	25 mL	3 × 25 mL	Room temperature (23±5°C)
Proteinase K (PK) Buffer	50 mL	3 × 50 mL	Room temperature (23±5°C)
Proteinase K (20 mg/mL)	1.25 mL	3 × 1.25 mL	Below –18°C

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

Overview of food testing

The kit is designed for preparation of DNA extraction from most food types. The kit procedure involves:

- Enrichment of food samples for *E. coli* O157:H7
- Nucleic acid extraction

To extract DNA from *E. coli* O157:H7 in food samples, the lysis protocol that is described on page 3 is recommended. The following sample volumes are recommended for the extraction of nucleic acid:

- From 25 g or 25 mL of food enriched for 6–8 hours (8–10 hours for juice), start with a 1-mL sample volume
- From 25 g or 25 mL of food enriched for 16–20 hours, start with a 200-µL sample volume
- From 375 g of food enriched for 16–20 hours, start with a 1-mL sample volume

In all cases, the recommended elution volume is 140 µL.

Preparation of reagents for first-time use

- **Binding Solution** – Add approximately 35 mL of 100% isopropanol to the empty Binding Solution bottle. Label the bottle to indicate that isopropanol is added.
- **Wash Buffer** – Add 74 mL of 95% ethanol to the Wash Buffer Concentrate bottle, mix well, then label the bottle to indicate that ethanol is added.

Before you begin

Before starting your sample extraction:

- Set the block heater temperature to 37°C.
- **Magnetic Particles** – Incubate the Magnetic Particles tube at 37±1°C for approximately 10 minutes, then vortex for approximately 10 seconds; keep at room temperature (23±5°C) until ready for use.

IMPORTANT! White precipitate occasionally forms in the Magnetic Particles tube. Extraction experiments show that formation of precipitate does not affect performance as long as the precipitate is redissolved and the Magnetic Particles are completely resuspended. Before using, always incubate the Magnetic Particles tube at 37±1°C for approximately 10 minutes, then vortex to completely resuspend.

Note: The Magnetic Particles are the limiting reagent in the PrepSEQ® Nucleic Acid Extraction Kit for Food Testing. Make sure that you have enough Magnetic Particles for the number of samples you will process. Either 25 or 30 µL of magnetic beads are required per sample depending on the workflow.

- Ensure that other reagents have been prepared as described in "Preparation of reagents for first-time use".
- For processing multiple samples in workflows that require Proteinase K treatment, we recommend preparing a Proteinase K Buffer mix:

  Premix 10 µL of Proteinase K (20 mg/mL) with 200 µL of PK 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 Proteinase K Buffer. Use immediately or store on ice until ready to use.

General differences between PrepSEQ® Nucleic Acid Extraction workflows

This document presents protocols for three different workflows, with general differences outlined below. To determine which protocol you should follow, consider the sample amount and select a workflow based on your preferred media and enrichment time.

Protocol	Sample amount	Media	Enrichment time†	Enrichment volume required for sample prep	Food type/ Proteinase K requirement	MagMAX™ Express-96 Magnetic Particle Processor script
Workflow A	25 g or 25 mL of food	Prewarmed BHI (Brain Heart Infusion)	6–8 hr (8–10 hr for juices)‡	1 mL§	Animal products‡‡: with Proteinase K	44000799DWPrepSEQGP
					Non-animal products: without Proteinase K	44000799DWPrepSEQGN
Workflow B	25 g or 25 mL of food	BPW (Buffered Peptone Water)	16–20 hr	200 µL††	Animal products: with Proteinase K (optional)	44000799DWPrepSEQPK
					Non-animal products: without Proteinase K	44000799DWPrepSEQDL
Workflow C	375 g of food	BPW	16–20 hr	1 mL§	All foods: with Proteinase K	44000799DWPrepSEQGP

IMPORTANT! Enrichment workflow C (sample amount = 375 g of food) was included in AOAC® validation studies but not in NF Validation™ certification (AFNOR) studies.

† All enrichments are incubated at 42±1°C.
‡ For convenience, samples can be enriched in BHI for up to 16 hours.
§ Pellet and resuspend in appropriate buffer.
†† Transfer directly to deep-well plate; no bacterial pellet required.
‡‡ Animal products include ground beef, beef trim, fish, and milk.

Kit workflow A: 6- to 8-hour enrichment for 25 g or 25 mL of food sample

E. coli O157:H7 enrichment

Step 1: Add 225 mL of prewarmed BHI to 25 g (or 25 mL) of food sample.

Step 2: Homogenize the food sample.

Step 3: Incubate at 42±1°C under static conditions for 6–8 hr (8–10 hr for juices).

Lysis protocol

Step 1: Transfer 1 mL of sample into a 1.5-mL tube.

Step 2: Centrifuge the tube at 12,000–16,000 × *g* for approximately 3 min.

Step 3: Remove and discard the supernatant as quickly as possible.

Step 4a: For animal products (with Proteinase K)

Add 210 µL of Proteinase K Buffer mix (10 µL PK + 200 µL PK Buffer). Mix well to resuspend the pellet.

Step 4b: For all other food types (without Proteinase K)

Add 300 µL of Lysis Buffer. Mix well to resuspend the pellet.

Step 5: Transfer the sample to a MagMAX™ Express-96 Deep Well Plate.

Step 6: Prepare the MagMAX™ Express-96 elution and wash plates.

Step 7a: For animal products (with Proteinase K)

Select **44000799DWPrepSEQGP** from the MagMAX™ Express-96 Magnetic Particle Processor.

Step 7b: For all other food types (without Proteinase K)

Select **44000799DWPrepSEQGN** from the MagMAX™ Express-96 Magnetic Particle Processor.

Step 8a–e: Load plates, then press **Start**.

Step 9: For animal products, proceed to step 10. For all other food types, proceed to step 12a-d.

Step 10: For animal products (with Proteinase K)

After 20 min, add 300 µL of Lysis Buffer to the lysis plate. Load the plate, then press **Start**.

Step 11: After 18 min, dispense the **Binding Mix**:

a. Vortex the Magnetic Particles for approximately 10 sec.
b. Add 30 µL of Magnetic Particles to each well.
c. Add 300 µL of Binding Solution to each well.
d. Load the plate into the instrument, then press **Start**.

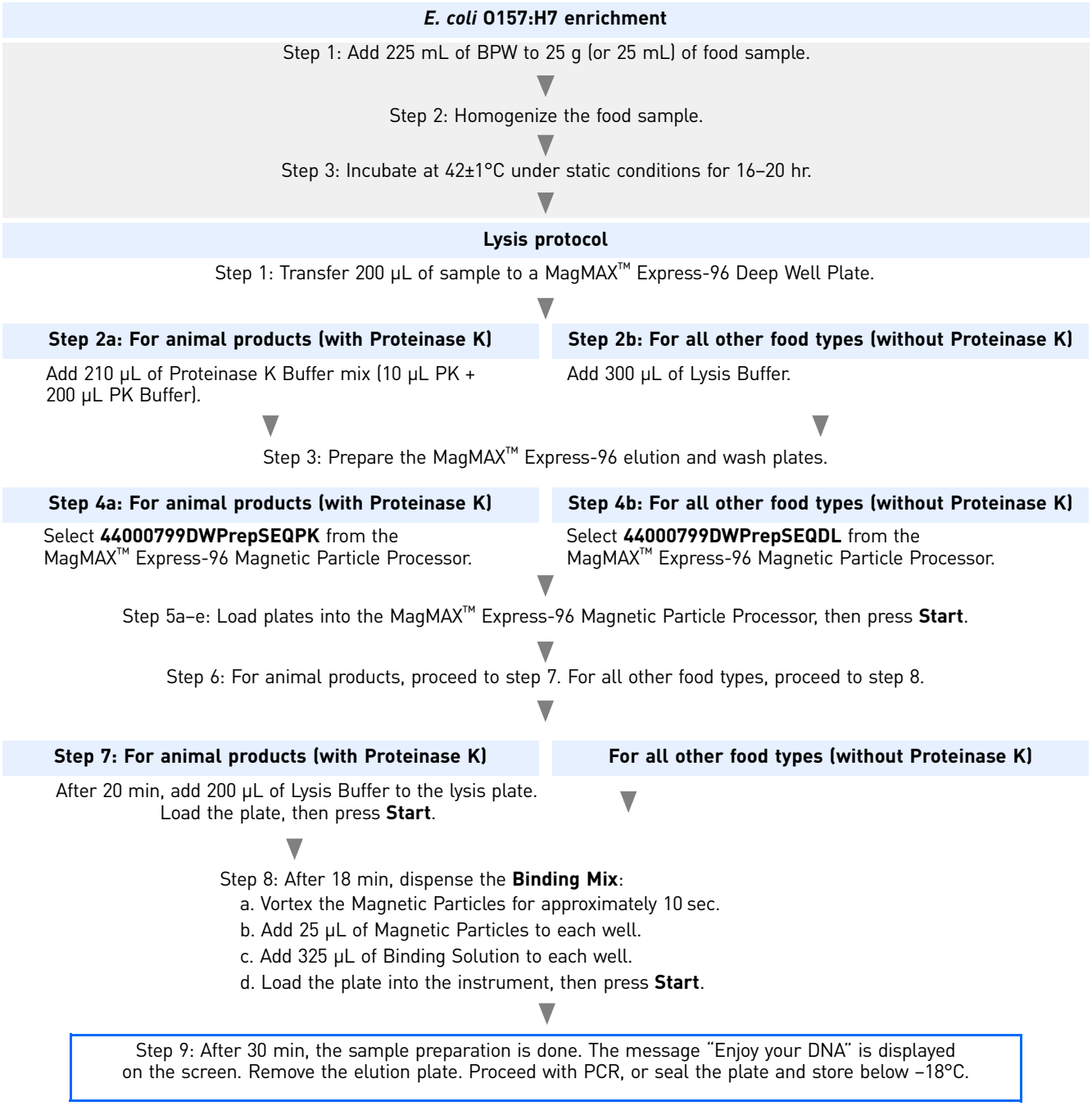
For all other food types (without Proteinase K)

Step 12: After 18 min, dispense the **Binding Mix**:

a. Vortex the Magnetic Particles for approximately 10 sec.
b. Add 30 µL of Magnetic Particles to each well.
c. Add 180 µL of Binding Solution to each well.
d. Load the plate into the instrument, then press **Start**.

Step 13: After 30 min, the sample preparation is done. The message "Enjoy your DNA" is displayed on the screen. Remove the elution plate. Proceed with PCR, or seal the plate and store below –18°C.

Kit workflow B: 16- to 20-hour enrichment for 25 g or 25 mL of food sample



Kit workflow C: 16- to 20-hour enrichment for 375 g of food sample

E. coli O157:H7 enrichment

Step 1: Add 1.5 L of BPW to 375 g of food sample.

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Step 2: Homogenize the food sample.

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Step 3: Incubate at 42±1°C under static conditions for 16–20 hr.

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Lysis protocol

Step 1: Transfer 1 mL of sample into a 1.5-mL tube.

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Step 2: Centrifuge the tube at 12,000–16,000 × *g* for approximately 3 min.

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Step 3: Remove and discard the supernatant as quickly as possible.

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Step 4: Add 210 µL of Proteinase K Buffer mix (10 µL PK + 200 µL PK Buffer). Mix well to resuspend the pellet.

Step 5: Transfer the sample to a MagMAX™ Express-96 Deep Well Plate.

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Step 6a–b: Prepare the MagMAX™ Express-96 elution and wash plates.

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Step 7: Select **44000799DWPrepSEQGP** from the MagMAX™ Express-96 Magnetic Particle Processor, then press **Start**.

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Step 8a – e: Load plates into the MagMAX™ Express-96 Magnetic Particle Processor, then press **Start**.

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Step 9: After 20 min, dispense the Lysis Buffer:

a. Add 300 µL of Lysis Buffer.

b. Load the plate into the instrument, then press **Start**.

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Step 10: After 18 min, dispense the **Binding Mix**:

a. Vortex the Magnetic Particles for approximately 10 sec.

b. Add 30 µL of Magnetic Particles to each well.

c. Add 300 µL of Binding Solution to each well.

d. Load the plate into the instrument, then press **Start**.

▼

Step 11: After 30 min, the sample preparation is done. The message "Enjoy your DNA" is displayed on the screen. Remove the elution plate. Proceed with PCR, or seal the plate and store below –18°C.

For testing of Food and Environmental samples only.

For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

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