USER GUIDE

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

For use with the MagMAX™ Express-96 Magnetic Particle Processor (Automated protocol)

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ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification

For testing of Food and Environmental samples only.

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TRADEMARKS:

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Product Information

IMPORTANT! Before using this product, read and understand the information in Appendix B, "Safety" on page 27.

CAUTION! *E. coli* O157:H7 is a Biosafety Level 2 (BSL-2) organism. Care must be taken when handling samples that may contain *E. coli* O157:H7. Laboratory personnel must be adequately trained to handle pathogens before being permitted to analyze samples for *E. coli* O157:H7. Laboratory personnel must wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. Extreme precautions should be taken with contaminated sharp items. Access to the laboratory should be limited when work is being conducted. Waste should be disposed of in compliance with local and national legislation as appropriate.

Product description

The PrepSEQ® Nucleic Acid Extraction Kit for Food Testing prepares high-quality microbial DNA and RNA from broth cultures using an automated sample preparation system, the MagMAX™ Express-96 magnetic particle processor (Cat. no. 4400078).

For additional background information, refer to Appendix A on page 25.

Materials and equipment

Kit contents

The PrepSEQ® Nucleic Acid Extraction Kit for Food Testing (Cat. no. 4428176) contains reagents for 300 reactions. Kit components and their storage conditions are shown in the table below. For information on the kit contents, refer to the "Materials supplied" section in the packaging insert for your kit.

Item	Quantity or volume	Storage
Lysis Buffer	6 bottles, 50 mL/bottle	Room temperature (23 ±5°C)
Magnetic Particles	6 tubes, 1.5 mL/tube	5 ±3°C
Binding Solution (Isopropanol)	3 empty bottles	Room temperature (23 ±5°C)
Wash Buffer Concentrate	6 bottles, 26 mL/bottle	Room temperature (23 ±5°C)
Elution Buffer	3 bottles, 25 mL	Room temperature (23 ±5°C)
Proteinase K (PK) Buffer	3 bottles, 50 mL	Room temperature (23 ±5°C)
Proteinase K (20 mg/mL)	3 tubes, 1.25 mL	Below –18°C

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

Materials not included in the kit

The following table includes materials and equipment for using (but not included in) the PrepSEQ® Nucleic Acid Extraction Kit. Unless otherwise indicated, many of the listed items are available from major laboratory suppliers (MLS).

Equipment, consumables, and reagents‡	
Item	**Source**
Equipment	
Block heater, 37°C	MLS
Benchtop microcentrifuge	Eppendorf 5415D or equivalent
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	Life Technologies Cat. no. 4400079
96-Well Magnetic-Ring Stand	Life Technologies Cat. no. AM10050
Homogenizer, Stomacher® 400 Laboratory Blender	Seward #0400/001/AJ or equivalent
Vortexer	MLS
Consumables	
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
MagMAX™ Express-96 Deep Well Tip Combs	Life Technologies Cat. no. 4388487
MagMAX™ Express-96 Deep Well Plates	Life Technologies Cat. no. 4388476
MagMAX™ Express-96 Standard Plates	Life Technologies Cat. no. 4388475
Whirl-Pak® Filter Bags, 10" × 15", 92 oz. (Stomacher® bags with mesh)	Nasco #B01488WA or equivalent
Whirl-Pak® Filter Bags, 6" × 9", 24 oz., 250/pkg (Stomacher® bags with mesh)	Nasco #B01348WA or equivalent
Whirl-Pak® Filter Bags, 6" × 9", 24 oz. (Stomacher® bags without mesh)	Nasco #B01297WA or equivalent
Microcentrifuge tubes, PCR clean, 1.5-mL	MLS
Reagents	
Brain Heart Infusion (BHI) Broth	MLS
Buffered Peptone Water (BPW)	MLS
Nuclease-free water	Life Technologies Cat. no. AM9938
Ethanol, 95%	MLS
Isopropanol, 100%	MLS

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

PrepSEQ® Nucleic Acid Extraction Kit for Food Testing: *E. coli* 0157:H7

Before you begin

Before starting your sample extraction:

- Set the block heater temperature to 37°C.
- Prepare the following reagents:

  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.

  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 dissolved prior to use. If a precipitate forms, incubate the tube at 37 ±1°C for approximately 10 minutes. If after 10 minutes the white precipitate is not completely dissolved, then longer incubation and higher temperatures (≤50°C) can be applied. Then vortex until completely resuspended.

  Note: The Magnetic Particles are the limiting reagent in the PrepSEQ® Nucleic Acid Extraction Kit. Make sure that you have enough Magnetic Particles for the number of samples you will process. Either 25 or 30 µL of magnetic beads per sample are required depending on the workflow (see workflows on pages 11, 12, and 13 for the volumes of Magnetic Particles required per sample).

- For processing multiple samples in workflows that require Proteinase K treatment, we recommend preparing a Proteinase K Buffer mix:
  a. Premix 10 µL of Proteinase K (20 mg/mL) with 200 µL of Proteinase K Buffer for each sample (use a clean appropriately sized container for mixing).
  b. Multiply volumes by the number of samples plus 10% for overage.
  c. Mix well to disperse Proteinase K in Proteinase K Buffer.
  d. Use immediately or store on ice until ready to use.
- Depending upon the workflow being followed, see the procedures that are described on pages 15, 18, or 20.

General differences between PrepSEQ® Nucleic Acid Extraction workflows

This document presents protocols for three different workflows, with general differences outlined in the following table. To determine which enrichment 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
Enrichment workflow A	25 g or 25 mL of food	Prewarmed Brain Heart Infusion (BHI)	6–8 hr (8–10 hr for juices)§	1 mL#	Animal products‡‡: with Proteinase K	44000799DWPrepSEQGP
					Non-animal products: without Proteinase K	44000799DWPrepSEQGN
Enrichment workflow B	25 g or 25 mL of food	Buffered Peptone Water (BPW)	16–20 hr	200 µL§§	Animal products: with Proteinase K (optional)	44000799DWPrepSEQPK
					Non-animal products: without Proteinase K	44000799DWPrepSEQDL
Enrichment 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 AFNOR validation 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.

‡‡ Animal products include ground beef, beef trim, fish, and milk.

§§ Transfer directly to deep-well plate; no bacterial pellet required.

Workflows

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

The following is a sample-preparation workflow for procedures starting on page 15.

***E. coli* O157:H7 enrichment** (page 14)

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 (page 15)

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 sterile microtiter 96-well deep well plate.
Step 6: Prepare the MagMAX™ Express 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 approx. 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 approx. 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 complete. 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: 16–20-hour enrichment for 25 g or 25 mL of food sample

The following is a sample-preparation workflow for procedures starting on page 18.

***E. coli* O157:H7 enrichment (page 14)**

Step 1: Add 225 mL of BPW 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 16–20 hr.

▼

Lysis protocol (page 18)

Step 1: Transfer 200 µL of sample to a sterile microtiter 96-well deep well plate.

▼

Step 2a: For animal products (with Proteinase K)
Add 210 µL of Proteinase K Buffer mix (10 µL PK + 200 µL PK Buffer).

▼

Step 2b: For all other food types (without Proteinase K)
Add 300 µL of Lysis Buffer.

▼

Step 3: Prepare the MagMAX™ Express elution and wash plates.

▼

Step 4a: For animal products (with Proteinase K)
Select **44000799DWPrepSEQPK** from the MagMAX™ Express-96 magnetic particle processor.

▼

Step 4b: For all other food types (without Proteinase K)
Select **44000799DWPrepSEQDL** from the MagMAX™ Express-96 magnetic particle processor.

▼

Step 5a–e: Load plates onto the MagMAX™ Express magnetic particle processor, then press Start.

Step 6: For animal products, proceed to step 7. For all other food types, proceed to step 8.

▼

Step 7: For animal products (with Proteinase K)
After 20 min, add 200 µL of Lysis Buffer to the lysis plate. Load the plate, then press Start.

▼

For all other food types (without Proteinase K)

▼

Step 8: After 18 min, dispense the **Binding Mix**:

a. Vortex the Magnetic Particles for approximately 10 sec.

b. Add 25 µL of Magnetic Particles to each well.

c. Add 325 µL of Binding Solution to each well.

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

▼

Step 9: After 30 min, the sample preparation is complete. 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 C: 16–20-hour enrichment for 375 g of food sample‡

The following is a sample-preparation workflow for procedures starting on page 20.

E. coli O157:H7 enrichment (page 14)

Step 1: Add 1500 mL of BPW to 375 g of food sample.

Step 2: Homogenize the food sample.

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

▼

Lysis protocol (page 20)

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 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 sterile microtiter 96-well deep well plate.

▼

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

▼

Step 7: Select **44000799DWPrepSEQGP** from the MagMAX™ Express-96 magnetic particle processor, then press **Start**.

▼

Step 8a–e: Load plates into the MagMAX™ Express-96 magnetic particle processor then press **Start**.

▼

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**.

▼

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 complete. 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.

‡ Not included in NF Validation studies

Protocol

Enrichment of food sample

1. Add to the food sample the appropriate enrichment media depending upon the *E. coli* O157:H7 workflow selected from the following options:
   - **Kit workflow A** – For 6–8-hour enrichment of 25 g or 25 mL of food (8–10-hour enrichment for juices), add 225 mL of prewarmed (42 ±1°C) Brain Heart Infusion (BHI) broth.
   - **Kit workflow B** – For 16–20-hour enrichment of 25 g or 25 mL of food, add 225 mL of Buffered Peptone Water (BPW).
   - **Kit workflow C** – For 16–20-hour enrichment of 375 g of food, add 1.5 L of BPW.

     Note: For 375-g food samples, use only the 16–20-hour enrichment.
2. Homogenize the food sample:
   - For 375-g food samples (Enrichment workflow C), use a filtered 10" × 15" Stomacher® bag (Nasco #B01488WA or equivalent) and squeeze the bag 5–10 times to break up food chunks.
   - For solid foods (such as chicken wings), use a filtered Stomacher® bag and mix by hand by squeezing the bag 5–10 times.
   - For coarse food types (such as meat, poultry, and seafood), use a filtered 6" × 9" Stomacher® bag (Nasco #B01348WA or equivalent) and stomach with the speed setting set to **Norm** for 1 minute (Stomacher® 400 Laboratory Blender or equivalent).
   - For soft food types (such as mayonnaise), use a standard 6" × 9" Stomacher® bag (Nasco #B01297WA or equivalent) and stomach with the speed setting set to **Norm** for 1 minute (Stomacher® 400 Laboratory Blender or equivalent).
   - For liquids or powdered foods, use a standard 6" × 9" Stomacher® bag (Nasco #B01297WA or equivalent) and mix by hand.
3. Incubate the food sample at 42 ±1°C under static conditions for the following amount of time:
   - **Kit workflow A** – 6–8 hours (8–10 hours for juices) to enable same-day result. For convenience, the sample can be enriched in BHI for up to 16 hours.
   - **Kit workflow B** – 16–20 hours.
   - **Kit workflow C** – 16–20 hours.

   Note: The minimum enrichment incubation time is 6 hours for Kit workflow A and 16 hours for Kit workflows B and C.

Next step

Proceed to the appropriate kit workflow as defined by your sample amount, enrichment time, and food type.

Protocol for use with workflow A: 6–8-hour enrichment for 25 g or 25 mL of food sample

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

1. Transfer 1 mL of sample into a 1.5-mL microcentrifuge tube.
2. Centrifuge the tube at 12,000–16,000 × *g* for approximately 3 minutes.
3. Remove and discard the supernatant as quickly as possible to prevent dissipation of pellet.

   Note: If no pellet is visible after centrifugation (for example, as found in filtered juices), leave ~50 µL of sample in the tube to avoid aspirating the bacterial pellet.

   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):

   1. Use a P1000 pipettor to remove fat from the top surface by aspirating in a circular motion without disturbing the pellet.
   2. Continue to collect supernatant from the top surface until all the supernatant is removed.
   3. Discard the supernatant into a waste container.

   or

   For solid fat layer (for example, as found in infant formula samples):

   1. Use a pipettor to gently dislodge the fat layer without disturbing the pellet.
   2. Pour off the supernatant and fat layer using a single quick motion.
   3. Aspirate the supernatant from the top surface using a pipettor until all the supernatant is removed.
   4. Discard the supernatant into a waste container.

4. Lyse the pellet:
   a. For animal products (such as ground beef, beef trim, fish, and milk), add 210 µl of Proteinase K Buffer mix (10 µL PK + 200 µL PK Buffer; see page 9). Mix well to resuspend the pellet.
   b. For all other food types, add 300 µL of Lysis Buffer. Mix well to resuspend the pellet.
5. Transfer the sample to a sterile microtiter 96-well deep-well (DW) plate (Cat no. 4388476).
6. Prepare the elution and wash plates:
   a. To prepare the elution plate, add 140 µL of Elution Buffer to those wells of the microtiter 96-well plate that correspond to the microtiter 96-well DW plate containing sample.
   b. To prepare wash plates 1 and 2, add 300 µL of Wash Buffer to those wells of the microtiter 96-well DW plate that correspond to the microtiter 96-well DW plate containing sample.

7. Select the script:
   - For animal products, select **44000799DWPrepSEQGP** program from the MagMAX™ Express-96 magnetic particle processor. Press **Start**.
   - For all other food types, select **44000799DWPrepSEQGN** program from the MagMAX™ Express-96 magnetic particle processor. Press **Start**.
8. Load the plates according to the readout. Verify orientation {A1 to A1}.
   a. **Tip combs** – In the microtiter 96-well plate; press **Start**.
   b. **Elution plate** (140 µL of Elution Buffer) – In the microtiter 96-well plate; press **Start**.
   c. **Wash plate 2** (300 µL of Wash Buffer) – In the microtiter 96-well DW plate; press **Start**.
   d. **Wash plate 1** (300 µL of Wash Buffer) – In the microtiter 96-well DW plate; press **Start**.
   e. **Lysis plate** (sample in PK Buffer mix or Lysis Buffer) – In the microtiter 96-well DW plate; press **Start**.
9. For animal products, proceed to step 10.
   or
   For all other food types, proceed to steps 12a–d.
10. For animal products using script 44000799DWPrepSEQGP *only*:
    After 20 minutes, the MagMAX™ Express-96 magnetic particle processor prompts you to dispense the Lysis Buffer. To dispense the Lysis Buffer:
    a. Add 300 µL of Lysis Buffer to each well.
    b. Load the plate into the instrument. Press **Start**.
11. For animal products using script 44000799DWPrepSEQGP *only*:
    After 18 minutes, the instrument prompts you to dispense the Binding Mix. To dispense the Binding Mix:
    a. Incubate the Magnetic Particles tube at 37 ±1°C for approximately 10 minutes, and then vortex for approximately 10 seconds until resuspension is complete.

    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 dissolved prior to use. If a precipitate forms, incubate the tube at 37 ±1°C for approximately 10 minutes. If after 10 minutes the white precipitate is not completely dissolved, then you may apply longer incubation and higher temperatures (≤50°C). Then vortex to completely resuspend.

    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. Press **Start**. Proceed to step 13.

12. For food types that are not of animal origin using script 44000799DWPrepSEQGN:

    a. Incubate the Magnetic Particles tube at 37 ±1°C for approximately 10 minutes, and then vortex for approximately 10 seconds until resuspension is complete.

    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 dissolved prior to use. If a precipitate forms, incubate the tube at 37 ±1°C for approximately 10 minutes. If after 10 minutes the white precipitate is not completely dissolved, then you may apply longer incubation and higher temperatures (≤50°C). Then vortex to completely resuspend.

    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. Press **Start**. Proceed to step 13.

13. When sample preparation is complete, 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.

IMPORTANT! Follow these tips when analyzing your DNA samples:

- For PCR, add 30 µL of eluate to the lyophilized assay. For information, refer to the *MicroSEQ® E. coli O157:H7 Detection Kit User Guide* (Pub. no. 4426511).
- If oil droplets are visible as a top layer in the elution plate samples, then collect eluate from the center of the tube (below the top layer; go to page 22 for instructions on pipetting eluate from food samples with high fat or oil content).
- If the elution plate contains magnetic beads, then place elution plate on a 96-well magnetic ring stand for ≥1 minute before collecting eluate for PCR.
- If the elution plate contains particulate residue from food sample that does not get removed using the 96-well magnetic ring stand, then centrifuge the elution plate at approximately 4000 × *g* for approximately 30 seconds to pellet particulate residue. Avoid particulate residue when collecting eluate for PCR.

Protocol for use with workflow B: 16–20-hour enrichment for 25 g or 25 mL of food sample

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

1. Transfer 200 µL of sample to a sterile microtiter 96-well deep well plate.
2. Lyse the sample:
   - For animal products (such as ground beef, beef trim, fish, and milk), add 210 µL of Proteinase K Buffer mix (10 µL PK + 200 µL PK Buffer; see page 9).
   - For all other food types, add 300 µl of the Lysis Buffer.
3. Prepare the elution and wash plates:
   a. To prepare the elution plate, add 140 µL of Elution Buffer to those wells of the microtiter 96-well plate that correspond to the microtiter 96-well DW plate containing sample.
   b. To prepare wash plates 1 and 2, add 300 µL of Wash Buffer to those wells of the microtiter 96-well DW plate that correspond to the microtiter 96-well DW plate containing sample.
4. Select the script:
   - For animal products, select **44000799DWPrepSEQPK** program from the MagMAX™ Express-96 magnetic particle processor. Press **Start**.
   - For all other food types, select **44000799DWPrepSEQDL** program from the MagMAX™ Express-96 magnetic particle processor. Press **Start**.
5. Load the plates into the MagMAX™ Express Magnetic Particle Processor according to the readout. Verify orientation {A1 to A1}.
   a. **Tip combs** – In the microtiter 96-well plate; press **Start**.
   b. **Elution plate** (140 µL of Elution Buffer) – In the microtiter 96-well plate; press **Start**.
   c. **Wash plate 2** (300 µL of Wash Buffer) – In the microtiter 96-well DW plate; press **Start**.
   d. **Wash plate 1** (300 µL of Wash Buffer) – In the microtiter 96-well DW plate; press **Start**.
   e. **Lysis plate** (sample in PK Buffer mix or Lysis Buffer) – In the microtiter 96-well DW plate; press **Start**.
6. For animal products, proceed to step 7.
   or
   For all other food types, proceed to step 8.
7. For animal products using script 44000799DWPrepSEQPK *only.*
   After 20 minutes, the MagMAX™ Express-96 magnetic particle processor prompts you to dispense the Lysis Buffer.
   a. Add 200 µL of Lysis Buffer to each well.
   b. Load the plate into the instrument. Press **Start**.

8. After 18 minutes, the MagMAX™ Express-96 magnetic particle processor prompts you to dispense the **Binding Mix**.

   a. Incubate the Magnetic Particles tube at 37 ±1°C for approximately 10ˆminutes, and then vortex for approximately 10 seconds until resuspension is complete.

   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 dissolved prior to use. If a precipitate forms, incubate the tube at 37 ±1°C for approximately 10 minutes. If after 10 minutes the white precipitate is not completely dissolved, then you may apply longer incubation and higher temperatures (≤50°C). Then vortex to completely resuspend.

   b. Add 25 µL of Magnetic Particles to each well.

   c. Add 325 µL of Binding Solution to each well.

   d. Load the plate into the instrument. Press **Start**.

9. When sample preparation is complete, the message "Enjoy your DNA" is displayed on the screen. Remove the elution plate. Store below –18°C.

IMPORTANT! Follow these tips when analyzing your DNA samples:

- For PCR, add 30 µL of eluate to the lyophilized assay. For information, refer to the *MicroSEQ® E. coli O157:H7 Detection Kit User Guide* (Pub. no. 4426511).
- If oil droplets are visible as a top layer in the elution plate samples, then collect eluate from the center of the tube (below the top layer; go to page 22 for instructions on pipetting eluate from food samples with high fat or oil content).
- If the elution plate contains magnetic beads, then place elution plate on a 96-well magnetic ring stand (Cat. no. AM10050) for ≥1 minute before collecting eluate for PCR.
- If the elution plate contains particulate residue from food sample that does not get removed using the 96-well magnetic ring stand, then centrifuge the elution plate at approximately 4000 × *g* for approximately 30 seconds to pellet particulate residue. Avoid particulate residue when collecting eluate for PCR.

Protocol for use with workflow C: 16–20-hour enrichment for 375 g of food sample

Note: Kit workflow C was not included in AFNOR validation studies.

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

1. Transfer 1 mL of sample into a 1.5-mL microcentrifuge tube.
2. Centrifuge the tube at 12,000–16,000 × *g* for approximately 3 minutes.
3. Remove and discard the supernatant as quickly as possible to prevent dissipation of pellet.

   Note: If no pellet is visible after centrifugation (for example, as found in filtered juices), leave ~50 µL of sample in the tube to avoid aspirating the bacterial pellet.

   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):

   1. Use a P1000 pipettor to remove fat from the top surface by aspirating in a circular motion without disturbing the pellet.
   2. Continue to collect supernatant from the top surface until all the supernatant is removed.
   3. Discard the supernatant into a waste container.

   or

   For solid fat layer (for example, as found in infant formula samples):

   1. Use a pipettor to gently dislodge the fat layer without disturbing the pellet.
   2. Pour off the supernatant and fat layer using a single quick motion.
   3. Aspirate the supernatant from the top surface using a pipettor until all the supernatant is removed.
   4. Discard the supernatant into a waste container.

4. Add 210 µL of Proteinase K Buffer mix (10 µL PK + 200 µL PK Buffer; see page 9). Mix well to resuspend the pellet.
5. Transfer the sample to a sterile microtiter 96-well deep well (DW) plate (Cat. no. 4388476).
6. Prepare the elution and wash plates:
   a. To prepare the elution plate, add 140 µL of Elution Buffer to those wells of the microtiter 96-well plate that correspond to the microtiter 96-well DW plate containing sample.
   b. To prepare wash plates 1 and 2, add 300 µL of Wash Buffer to those wells of the microtiter 96-well DW plate that correspond to the microtiter 96-well DW plate containing sample.
7. Select **44000799DWPrepSEQGP** program from the MagMAX™ Express-96 magnetic particle processor. Press Start.

8. Load the plates into the MagMAX™ Express-96 magnetic particle processor according to the readout. Verify orientation {A1 to A1}.
   a. **Tip combs** – In the microtiter 96-well plate; press **Start**.
   b. **Elution plate** (140 µL of Elution Buffer) – In the microtiter 96-well plate; press **Start**.
   c. **Wash plate 2** (300 µL of Wash Buffer) – In the microtiter 96-well DW plate; press **Start**.
   d. **Wash plate 1** (300 µL of Wash Buffer) – In the microtiter 96-well DW plate; press **Start**.
   e. **Lysis plate** (sample in PK Buffer mix) – In the microtiter 96-well DW plate; press **Start**.
9. After 20 minutes, the MagMAX™ Express-96 magnetic particle processor prompts you to dispense the Lysis Buffer.
   a. Add 300 µL of Lysis Buffer to each well.
   b. Load the plate into the instrument. Press **Start**.
10. After 18 minutes, the instrument prompts you to dispense the **Binding Mix**.
    a. Incubate the Magnetic Particles tube at 37 ±1°C for approximately 10 minutes, then vortex for approximately 10 seconds until resuspension is complete; 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 dissolved prior to use. If a precipitate forms, incubate the tube at 37 ±1°C for approximately 10 minutes. If after 10 minutes the white precipitate is not completely dissolved, then you may apply longer incubation and higher temperatures (≤50°C). Then vortex to completely resuspend.

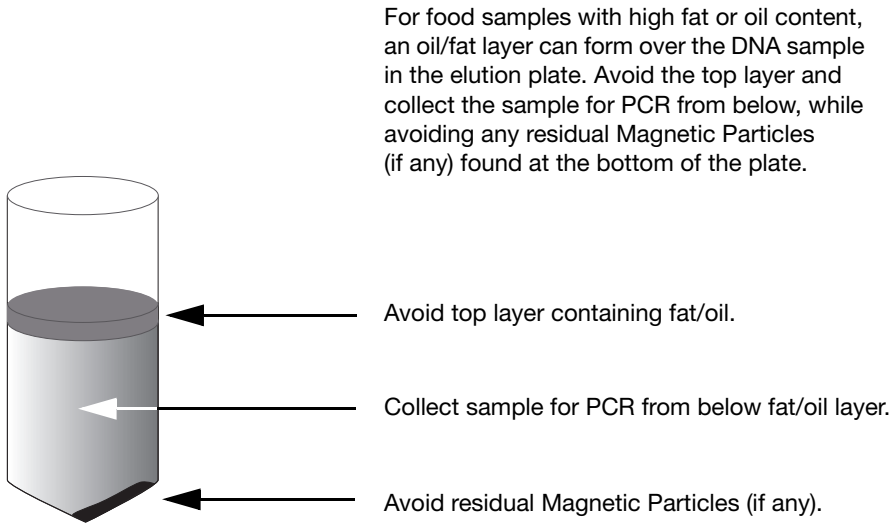
    b. Add 30 µL of Magnetic Particles to each well.
    c. Add 300 µL of Binding Solution to each well.
    d. Load the plate into the instrument. Press **Start**.

11. When the sample preparation is complete, the message "Enjoy your DNA" is displayed on the screen. Remove the elution plate. Store below –18°C.

IMPORTANT! Follow these tips when analyzing your DNA samples:

- For PCR, add 30 µL of eluate to the lyophilized assay. For information, refer to the *MicroSEQ® E. coli O157:H7 Detection Kit User Guide* (Pub. no. 4426511).
- If oil droplets are visible as a top layer in the elution plate samples, then collect eluate from the center of the tube (below the top layer; see the following figure for instructions on pipetting eluate from food samples with high fat or oil content).
- If the elution plate contains magnetic beads, then place elution plate on a 96-well magnetic ring stand (Cat. no. AM10050) for ≥1 minute before collecting eluate for PCR.
- If the elution plate contains particulate residue from food sample that does not get removed using the 96-well magnetic ring stand, then centrifuge the elution plate at approximately 4000 × *g* for approximately 30 seconds to pellet particulate residue. Avoid particulate residue when collecting eluate for PCR.

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



Troubleshooting

For food testing

Observation	Possible cause	Recommended action
Inhibition of PCR is indicated by non-detection of IPC reaction.	Magnetic Particles were in the elution plate.	Avoid disturbing the Magnetic Particles during transfer of eluted DNA to the lyophilized assay. *Optional:* Spin the plate at approximately 4000 × *g* for approximately 30 seconds to pellet the Magnetic Particles to the bottom of the plate. *or* Place the elution plate on the 96-Well Magnetic Ring Stand (Cat. no. AM10050) during transfer of sample to the lyophilized assay.
	Elution plate contains incompletely removed particulate residue from food sample.	Avoid residue during transfer of eluted DNA to lyophilized assay. *Optional:* Spin the plate at approximately 4000 × *g* for approximately 30 seconds to pellet the food residue to the bottom of the plate.
	Removal of sample supernatant before addition of Lysis Buffer was incomplete.	Ensure maximal removal of the supernatant without disturbing the bacterial pellet.
The bacterial pellet separates from the tube, making the pellet difficult to avoid during aspiration.	The sample was left unattended before aspirating off the supernatant, causing dissipation of the bacterial pellet.	Re-centrifuge and remove the supernatant immediately following centrifugation.
	The size of the bacterial pellet is very small and difficult to see.	Remove the supernatant carefully, leaving behind up to 50 µL of supernatant, to avoid aspiration of pellet.
The bacterial pellet is difficult to resuspend.	Pellet is too hard.	Ensure maximum resuspension of the pellet in the Lysis Buffer or Proteinase K Buffer before proceeding.
		Transfer the entire contents, including the incompletely resuspended pellet (if any) to the Lysis Plate.

A Background Information

Overview

Use the PrepSEQ® Nucleic Acid Extraction Kit to prepare food samples to test for *E. coli* O157:H7. 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 (automated with the MagMAX™ Express-96 magnetic particle processor)

To extract DNA from *E. coli* O157:H7 in food samples, we recommend the lysis protocol that is described on page 15. 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.

AOAC Performance Tested Methods℠ Certification

The MicroSEQ® *E. coli* O157:H7 Detection Kit earned the Performance Tested Methods℠ Certification from the AOAC Research Institute. The validation was conducted using USDA MLG 5.04 as the reference method for meat products, and ISO 16654 as the reference method for leafy green products and juices. The validated workflow includes:

- Two sample preparation kit options:
  - PrepSEQ® Nucleic Acid Extraction Kit
  - PrepSEQ® Rapid Spin Sample Preparation Kit
- MicroSEQ® *E. coli* O157:H7 Detection Kit
- Applied Biosystems® 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software

The method was certified for use with the following matrices:

- 25 g of ground beef and beef trim
- 375 g of ground beef and beef trim
- 25 g of spinach
- 25 g of apple juice
- 25 g of orange juice

ISO 16140 Validation

ABI 29/03 – 03/11
[End of validity: refer to certificate at www.afnor-validation.com]
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification

The MicroSEQ® *E. coli* O157:H7 Detection Kit was certified "NF Validation". The ISO 16140 standard was used for the validation of alternative methods. This kit was compared and found equivalent to the ISO 16654 reference method. The validated workflow includes:

- Two sample preparation kit options:
  - PrepSEQ® Nucleic Acid Extraction Kit
  - PrepSEQ® Rapid Spin Sample Preparation Kit
- MicroSEQ® *E. coli* O157:H7 Detection Kit
- Applied Biosystems® 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software

The method, using workflows A or B, was certified for use with the following matrices: raw beef meat and raw produce.

General recommendations:

- Comply with Good Laboratory Practices – GLP (Refer to EN ISO 7218 standard).
- In the context of NF Validation, sample sizes of more than 25 gram have not been tested.
- ISO 16654 and ISO 7218 are recommended for preparation of master suspensions.

For more information about the expiration date of the "NF Validation" certification, please refer to the certificate, available on the website at: **www.afnor-validation.com**

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.

Specific chemical handling

CAS	Chemical	Phrase
50-01-1	Guanidine HCl	Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid wastes containing this product.
593-84-0	Guanidine Isothiocyanate	Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid wastes containing this product.

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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

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 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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