USER GUIDE

applied biosystems® by life technologies™

Pathatrix® 5-Pooling *E. coli* O157:H7 Kit Linked to PCR

For use with the Pathatrix® Auto Instrument

Catalog Number APE250SDP

Publication Number MAN0007094
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For testing of Food and Environmental samples only.

life technologies™

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Product Information

IMPORTANT! Before using this product, read and understand the information in Appendix C, "Safety" on page 50.

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.

About the kit

The Pathatrix® 5-Pooling *E. coli* O157:H7 Kit provides a sample preparation method for presence/absence testing based on the detection of as few as 1–10 cfu (colony forming units)/25–375 g of food sample. An AOAC-validated protocol for using this kit for sample preparation followed by detection of microorganisms by selective agar plates can be downloaded from **www.lifetechnologies.com/pathatrix** (Pathatrix® 5-Pooling *E. coli* O157:H7 Kit Linked to Selective Agar Plates, Pub. no. MAN0007090).

This user guide provides 4 alternative protocols for using the Pathatrix® 5-Pooling *E. coli* O157:H7 Kit followed by detection of microorganisms by PCR. Presumptive results can be obtained within the following times:

- When linked to the DuPont® BAX® PCR system:
  - 8–11 hours (same-day–enrichment protocol; Chapter 1)
  - 20–23 hours (overnight-enrichment protocol; Chapter 2)
- When linked to the Idaho Technology R.A.P.I.D.® LT PCR system:
  - 7–9 hours (same-day–enrichment protocol; Chapter 3)
  - 18–21 hours (overnight-enrichment protocol; Chapter 4)

A presumptive positive isolate should be subsequently confirmed by the use of subculture, as well as appropriate biochemical and serological tests as required.

Once confirmed, the results are reported as:

- *E. coli* O157:H7 **Detected** in 25–375 g (sample matrices)
- *E. coli* O157:H7 **Not detected** in 25–375 g (sample matrices)

See Appendix A on page 47 for additional background information.

Kit contents

The Pathatrix® 5-Pooling *E. coli* O157:H7 Kit (Cat. no. APE250SDP) contains enough consumable components and Pathatrix® paramagnetic beads to process 250 samples (50 Cartridge runs).

Item	Quantity or volume	Storage
Pre-sterilized Sample and Elution Vessel Packs	50 each	Room temperature
Pre-sterilized Capture Phase Packs	50 each	Room temperature
Pre-sterilized Flat Cap Lids	50 each	Room temperature
Anti-*E. coli* O157:H7 Antibody-Coated Paramagnetic Beads†	2.5 mL (50 tests)	5 ±3°C

† The beads have a shelf life of 12 months and are labeled with an expiration date accordingly.

IMPORTANT! Never freeze the Pathatrix® paramagnetic bead suspension. Beads that have been subjected to freezing temperatures may be rendered inactive.

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

Item	Source
Equipment	
Incubator, 37 ±1°C	MLS
Incubator, 42 ±1°C	MLS
Pathatrix® Auto Instrument	Life Technologies Cat. no. PATHATRIXAUTO
For use with the BAX® PCR protocol: Magnetic Capture Plate	Life Technologies Cat. no. MAGNETICPLATE
For use with the R.A.P.I.D.® LT PCR protocol and VIDAS® protocol: DynaMag™-2 Magnet	Life Technologies Cat. no. 123.21D
Forceps, scissors, spatula, knife, and/or scalpel	MLS

Item	Source
Consumables	
Sterile bags for enrichment (Whirl-Pak® or Stomacher® bag, or equivalent)	Nasco # B01196WA, Seward product code BA6041, or equivalent
Optional for high-particulate or high-fat-content samples: • Sterile filter bags for enrichment (Whirl-Pak® or Stomacher® bag, or equivalent) ***or*** • Pathatrix® Foam filters • Pathatrix® 5-Pool Kit – Straws (254 mm) and Syringes (10 mL)	 • Nasco # B01348WA, Seward product code BA6041/STR, or equivalent ***or*** • Life Technologies Cat. no. PFF • Life Technologies Cat. no. POOL510MLN
Microcentrifuge tubes, PCR clean, 1.5-mL	MLS
Sterile 10-µL disposable loops	MLS
Media	
See Sample Enrichment sections in the protocols (page 12, 20, 28, or 38) for recommendations about enrichment media choice. The media is supplied by several manufacturers (e.g., Oxoid [product codes shown], Difco, and Merck) in a dehydrated form and should be prepared according to the manufacturer's instructions.	
Buffered peptone water	Oxoid product code CM0509
Buffered peptone water (ISO formulation) (alternative)	Oxoid product code CM1049
Selective agar	
For CT-SMAC plates: • Sorbitol MacConkey agar • C-T Supplement	 • Oxoid product code CM0813B • Oxoid product code SR0172E
BBL CHROMagar O157	BD Catalog # 214984
Reagents	
PBS, 10X, pH 7.4	Life Technologies Cat. no. AM9624 or AM9625

1 Pathatrix® Kit Linked to the DuPont® BAX® PCR System – Same-Day–Enrichment Format

Workflow

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Procedural guidelines

- Use aseptic technique and good laboratory practices at all times.
- A facemask should be worn when weighing out powders.
- Care must be taken when boiling agar prior to autoclaving, and heat-resistant gloves should be worn when handling hot flasks of liquid.
- Take care when handling plates or tubes that contain microorganisms.
- Avoid generating aerosols, as pathogenic organisms may be present.
- Used or unused reagents, used media, sample enrichments, as well as other contaminated disposable materials should be disposed of following procedures for infectious or potentially infectious products.
- Sample enrichments might be contaminated with pathogenic organisms infectious to humans, so all waste must be treated as biohazardous and handled and disposed using safe laboratory practices, in accordance and compliance with all appropriate regulations.

Sample enrichment

1. Weigh the food sample (typically 25–375 g) into an appropriate sterile bag.
   - For sample sizes between 25–55 g, prepare a 1:10 dilution of the food sample in **pre-warmed (42 ±1**°C**)** Buffered Peptone Water. For example, add 25 g of food sample to 225 mL of prewarmed media.
   - Sample sizes above 55 g should be diluted in 500 mL of pre-warmed Buffered Peptone Water.

   IMPORTANT! It is critical that the enrichment media is prewarmed to 42 ±1°C prior to adding the food sample. To prevent cooling, all samples should then immediately be placed in the incubator at 42 ±1°C.

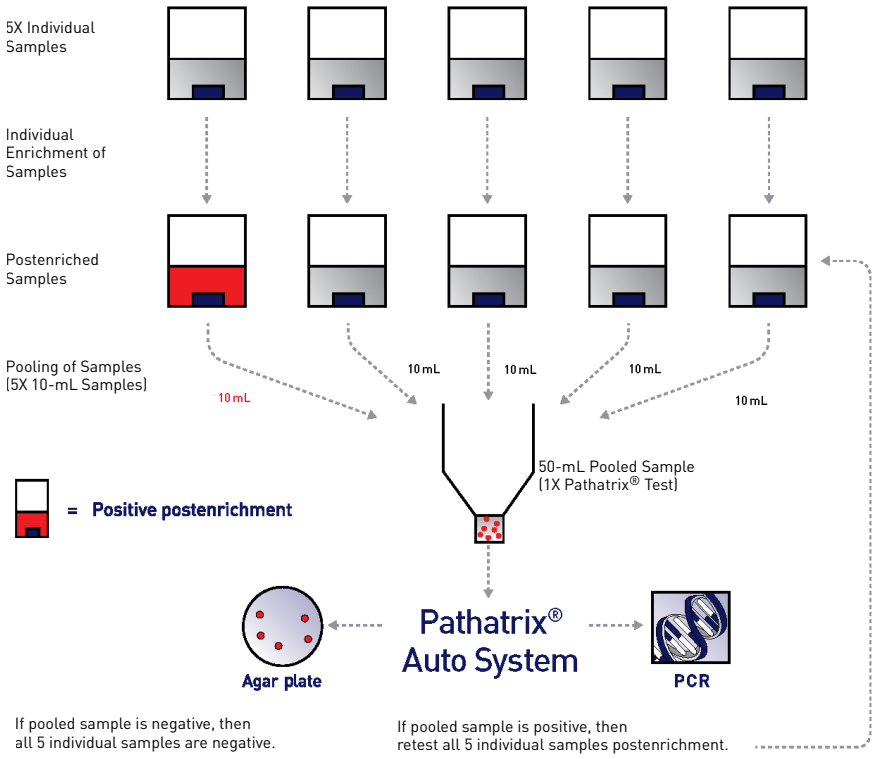
2. Homogenize the sample by massaging the sample between the fingers for 10–15 seconds to disperse any large clumps of material (Narang and Cray, 2006).
3. Incubate at **42 ±1**°C for a **minimum** of **6 hours**.

   Note: We recommend that these sub-samples for pooling are processed immediately or, if storage is required, that the samples are refrigerated at 5 ±3°C. Samples should be rewarmed to 37 ±1°C prior to analysis on the Pathatrix® Auto Instrument. The remaining enriched sample should be stored at 5 ±3°C for up to 32 hours until the results of the pooled sample have been determined.

Sample preparation

1. Remove the Sample Vessel and Elution Vessel from the consumable kit packaging and place into the Sample Vessel Holder.
2. Partially remove the lids from both vessels, making a large enough opening to allow the sample and wash buffer to be dispensed into the vessels.
3. Prepare a single, pooled sample in the Sample Vessel by pooling **5 × 10-mL** aliquots from 5 individually enriched samples to create a 50-mL pooled sample (see the following figure).

   Note: If the samples are highly particulate and/or contain a high fat content, the use of the FiltaFoam system (Foam filters, Cat. no. PFF) with pooling syringes and straws (Cat. no. POOL510MLN) is recommended. Alternatively, Seward plain sterile bags with internal strainers may be used (Seward Product Code BA6041/STR).

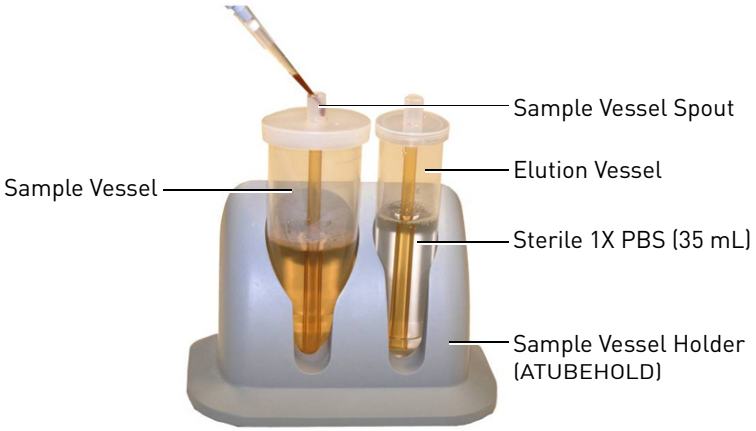


4. Store the individual enriched samples at 5 ±3°C for potential reanalysis until the test result is confirmed.

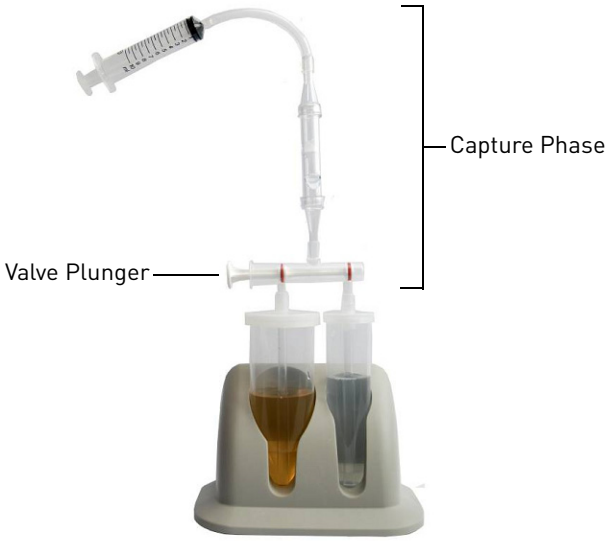
   Note: Do not store for more than 32 hours.

Samples and consumable loading

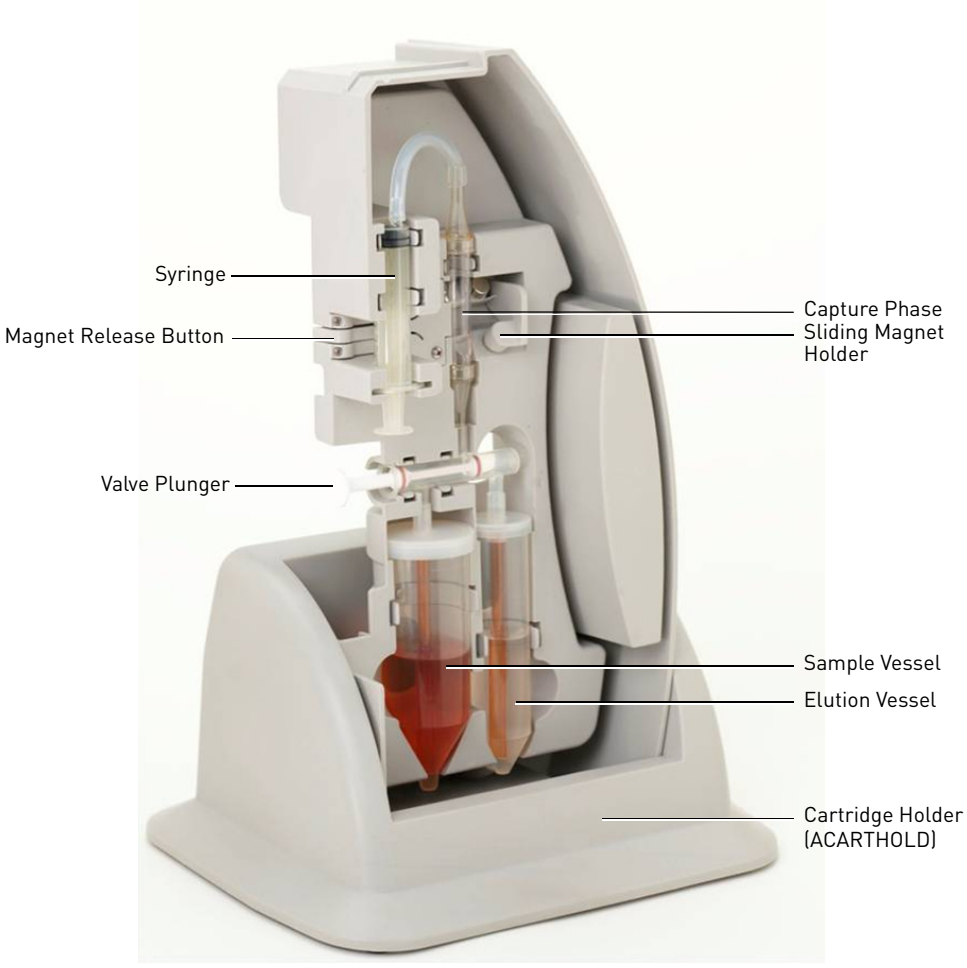
1. Add PBS (Cat. no. AM9624, diluted to 1X) to the fill line of the Elution Vessel (approximately 35 mL of 1X PBS).
2. Place the lids on the Sample and Elution Vessels, making sure that the vessels are sealed all the way around.
3. Ensure the Pathatrix® paramagnetic beads (included in the kit) are fully resuspended by agitating the bead vial (for example, by vortexing bead vial or inversion of sealed vial), and add 50 µL of the Pathatrix® paramagnetic bead suspension into the spout on the lid of the Sample Vessel.



4. Remove the capture-phase kit from the bag and orient with the valve plunger pointing left. Connect the valve firmly to the lids of the Sample and Elution Vessels.



5. Holding both vessels, lift the assembled vessels and attached capture-phase kit out of the Sample Vessel Holder.
6. Place the vessels into the Cartridge, pushing them in firmly from the bottom upwards.
7. Firmly push the rest of the kit into the Cartridge, ensuring that the valve, the capture phase, and the syringe are all held securely in the molded recess of the Cartridge.
8. Push the magnet slider across into the locking position and press the magnet release button on the side of the Cartridge to ensure the kit is positioned correctly and the magnets can freely disengage from the capture phase.



9. Reset the magnets into the locking position.
10. Insert the Cartridge into the Pathatrix® Auto Instrument until it clicks into the locking position.
11. Press the numbered button above the appropriate Cartridge to start the run (approximately 14 minutes). The associated LED will turn green to indicate the run has started.

Sample unloading

1. At the end of the run, the LED will flash red and green alternately.
2. Press the button above the appropriate Cartridge to initialize the draining step (approximately 1 minute).
3. When the draining step is complete and the LED is illuminated red, remove the Cartridge by pulling it out, away from the instrument.
4. Remove the syringe from the Cartridge and carefully pull out the rest of the kit, starting at the top and working downwards. When removing the Sample and Elution Vessels from the Cartridge, hold firmly by the vessels themselves to prevent spillage.
5. Place both vessels in the Tube Rack (also known as the Sample Vessel Holder or rack).
6. Remove the lid from the Elution Vessel and, leaving the Elution Vessel in place in the rack, lift away the rest of the consumable, including the Sample Vessel, and discard.
7. Place the Elution Vessel in the rack and cap with a flat lid (provided in the kit). Leave in the rack for 1 minute to allow capture of the Pathatrix® paramagnetic beads.
8. Remove all the liquid from the Elution Vessel, without removing the vessel from the rack, taking care not to disturb the captured Pathatrix® paramagnetic beads.
9. Remove the Elution Vessel from the vessel holder, add 100 µL of PBS into the Elution Vessel, and resuspend the Pathatrix® paramagnetic beads.
10. Appropriate aliquots of the Pathatrix® paramagnetic bead suspension can then be immediately analyzed using the laboratory's chosen pathogen detection method.

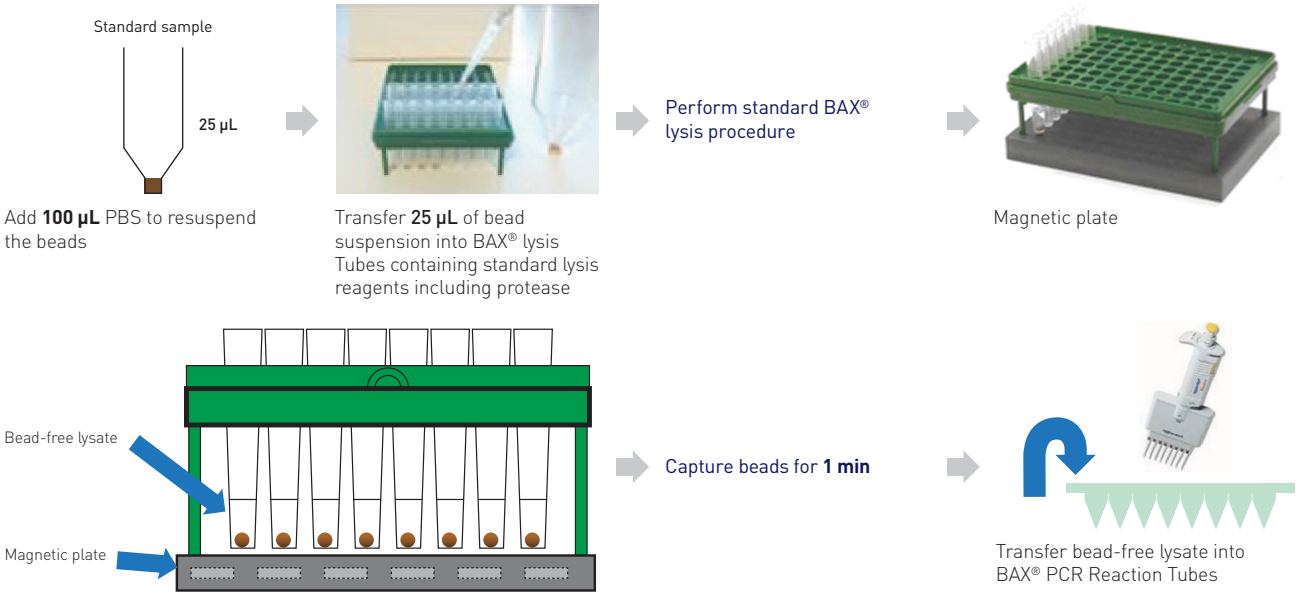
Note: The Pathatrix® paramagnetic bead suspension may be retained for later testing if necessary. The beads should be stored in 1.5-mL microcentrifuge tubes AWAY from magnets (for example, away from Pathatrix® vessel holders) at 5 ±3°C for up to 24 hours.

Detection – BAX® PCR protocol

IMPORTANT! It is solely the operator's responsibility to refer to the package insert instructions and instrument user manual for information regarding the correct set up and operation of the BAX® PCR system.

1. Pipet 25 µL of the Pathatrix® paramagnetic bead suspension into BAX® lysis tubes and proceed according to standard BAX® lysis procedure.
2. Once the lysis step is complete, immediately place the Rack and Tubes onto the Magnetic Capture Plate (Cat. no. MAGNETICPLATE) and leave for at least 1 minute to allow the Pathatrix® paramagnetic beads to accumulate on the bottom of the tube.
3. Transfer only **bead-free lysate** into the BAX® PCR reaction tubes

   Note: Target DNA, if present, will be in the **bead-free** supernatant.
4. To proceed, refer to the DuPont® Qualicon operating instructions.



If a negative result is obtained from the pooled sample, the individual enrichments can be discarded, as further testing is not required.

If a positive result is obtained from the pooled sample, the individual enrichments can be retested to allow identification of which individual samples in the pool produced the positive result (see the following section, "Retesting of individual samples after "Positive Pooled Sample"").

Retesting of individual samples after "Positive Pooled Sample"

1. Individual samples, which require retesting, **should be rewarmed to 37 ±1°C** before having **10 mL** removed and transferred into a Sample Vessel.
2. Individual samples should be retested immediately by repeating all the steps in Samples and consumable loading through Detection – BAX® PCR protocol described above.

If a positive PCR result is subsequently obtained, an aliquot of the Pathatrix® paramagnetic beads should be plated out (see the following section, "Detection – direct plating (optional)").

Detection – direct plating (optional)

Note: We recommend streaking the sample out to generate individual colonies, as opposed to spread plating.

1. Pipet all of the remaining Pathatrix® paramagnetic bead suspension onto the edge of well-dried selective agar plates (for example, Cefixime Potassium Tellurite Sorbitol-MacConkey Agar [CT-SMAC], CHROMagar O157).

   Note: Retain an amount of bead suspension you wish to keep. Divide the remainder of the bead suspension into equal amounts for streaking on your selective agar plates.
2. Using a sterile 10-µL inoculation loop, streak from this pool to generate isolated colonies.
3. Allow the plates to dry for approximately 10 minutes then invert and incubate at at the required temperature for 18–24 hours.

A presumptive positive result is defined as the isolation of typical, suspicious, or atypical *E. coli* O157:H7 colonies on the agar plates used. A presumptive positive isolate should be subsequently confirmed by the use of subculture, appropriate biochemical and serological tests (for example, as detailed in ISO 16654:2001 or USDA Microbiology Laboratory Guidebook [MLG] 5.04 as used in the AOAC Research Institute validation study [See "References" on page 53]).

Test result interpretation and classification

The Pathatrix® 5-Pooling *E. coli* O157:H7 Kit is designed as a sample preparation method for presence/absence detection of *E. coli* O157:H7 in food matrices

Using the Pathatrix® 5-Pooling *E. coli* O157:H7 Kit (same-day–enrichment format) linked to the BAX® PCR system, presumptive results can be obtained, prior to confirmation, within 8–11 hours.

Once confirmed, the results are reported as:

- *E. coli* O157:H7 **Detected** in 25–375 g (sample matrices)
- *E. coli* O157:H7 **Not detected** in 25–375 g (sample matrices)

2 Pathatrix® Kit Linked to the DuPont® BAX® PCR System – Overnight-Enrichment Format

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Test result interpretation and classification (page 26)

Procedural guidelines

- Use aseptic technique and good laboratory practices at all times.
- A facemask should be worn when weighing out powders.
- Care must be taken when boiling agar prior to autoclaving, and heat-resistant gloves should be worn when handling hot flasks of liquid.
- Take care when handling plates or tubes that contain microorganisms.
- Avoid generating aerosols, as pathogenic organisms may be present.
- Used or unused reagents, used media, sample enrichments, as well as other contaminated disposable materials should be disposed of following procedures for infectious or potentially infectious products.
- Sample enrichments might be contaminated with pathogenic organisms infectious to humans, so all waste must be treated as biohazardous and handled and disposed using safe laboratory practices, in accordance and compliance with all appropriate regulations.

Sample enrichment

1. Weigh the food sample (typically 25–375 g) into an appropriate sterile bag.
   - For sample sizes between 25–55 g, prepare a 1:10 dilution of the food sample in **pre-warmed (42 ±1**°C**)** Buffered Peptone Water. For example, add 25 g of food sample to 225 mL of prewarmed media.
   - Sample sizes above 55 g should be diluted in 500 mL of pre-warmed Buffered Peptone Water.

   IMPORTANT! It is critical that the enrichment media is prewarmed to 42 ±1°C prior to adding the food sample. To prevent cooling, all samples should then immediately be placed in the incubator at 42 ±1°C.

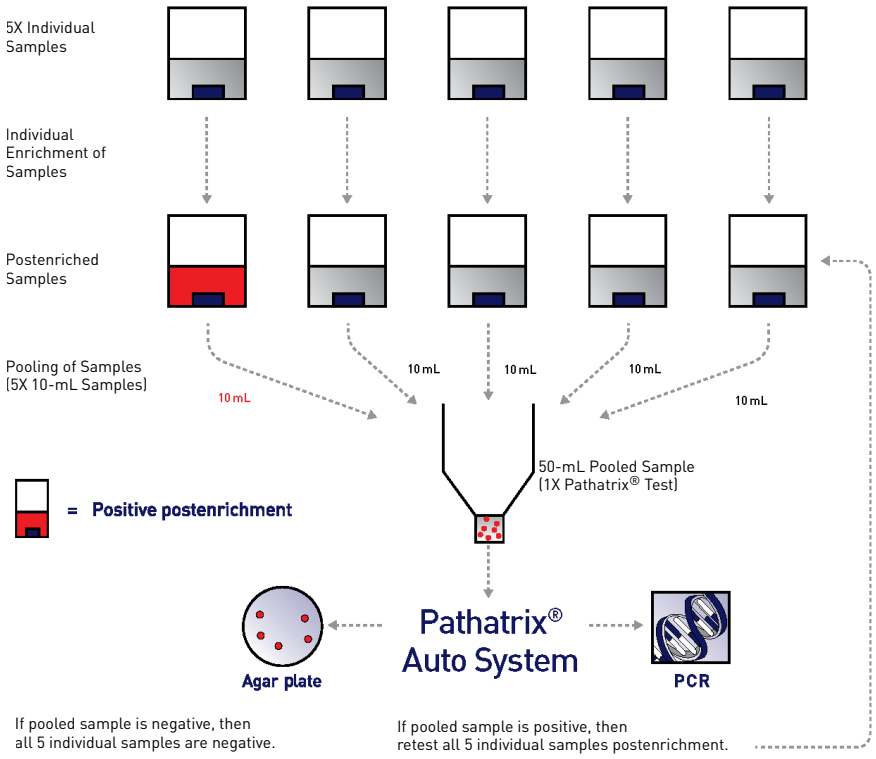
2. Homogenize the sample by massaging the sample between the fingers for 10–15 seconds to disperse any large clumps of material (Narang and Cray, 2006).
3. Incubate at **42 ±1**°C for a **minimum** of **16 hours** up to a **maximum** of **24 hours**.

   Note: We recommend that these sub-samples for pooling are processed immediately or, if storage is required, that the samples are refrigerated at 5 ±3°C. Samples should be rewarmed to 37 ±1°C prior to analysis on the Pathatrix® Auto Instrument. The remaining enriched sample should be stored at 5 ±3°C for up to 32 hours until the results of the pooled sample have been determined.

Sample preparation

1. Remove the Sample Vessel and Elution Vessel from the consumable kit packaging and place into the Sample Vessel Holder.
2. Partially remove the lids from both vessels, making a large enough opening to allow the sample and wash buffer to be dispensed into the vessels.
3. Prepare a single, pooled sample in the Sample Vessel by pooling **5 × 10-mL** aliquots from 5 individually enriched samples to create a 50-mL pooled sample (see figure below).

   Note: If the samples are highly particulate and/or contain a high fat content, the use of the FiltaFoam system (Foam filters, Cat. no. PFF) with pooling syringes and straws (Cat. no. POOL510MLN) is recommended. Alternatively, Seward plain sterile bags with internal strainers may be used (Seward Product Code BA6041/STR).

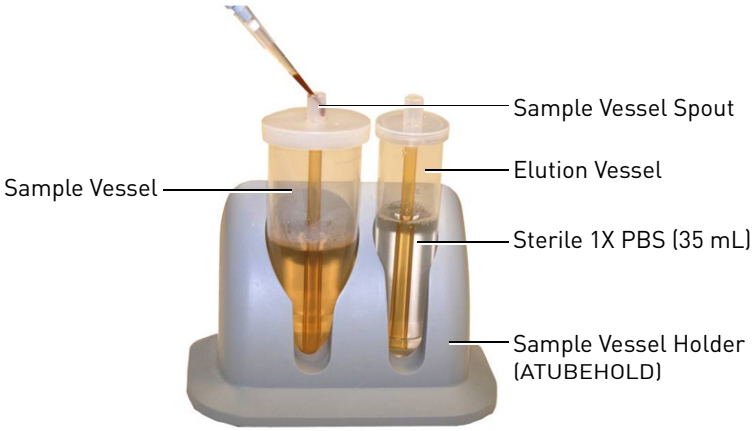


4. Store the individual enriched samples at 5 ±3°C for potential reanalysis until the test result is confirmed.

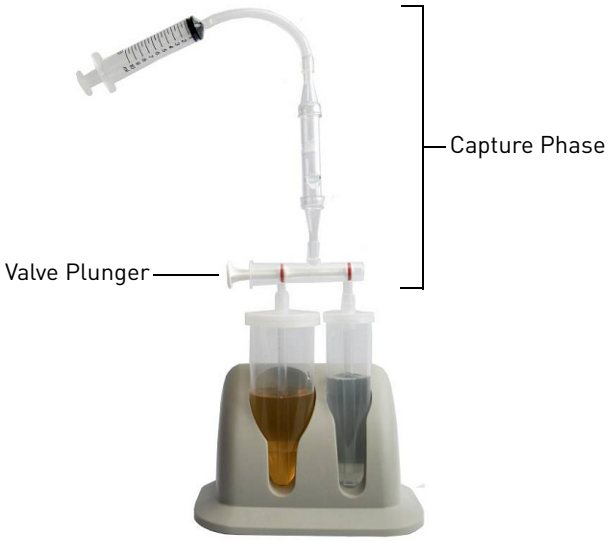
   Note: Do not store for more than 32 hours.

Samples and consumable loading

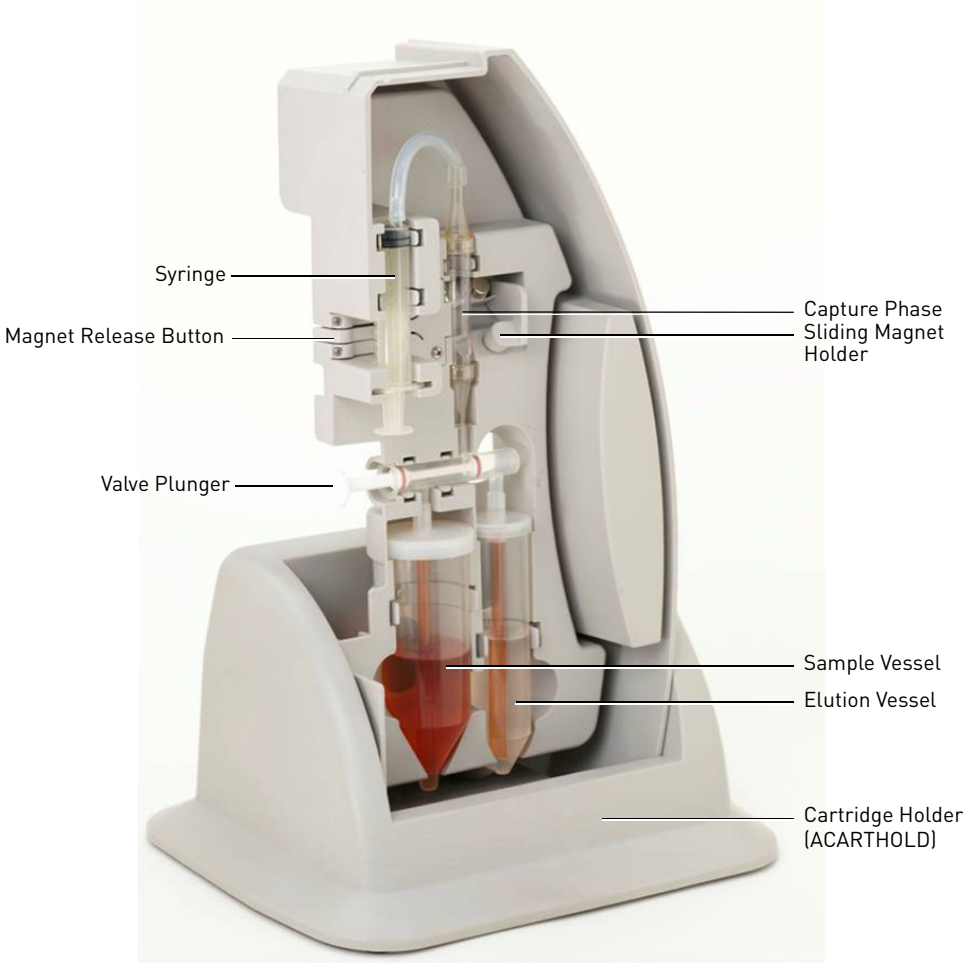
1. Add PBS (Cat. no. AM9624, diluted to 1X) to the fill line of the Elution Vessel (approximately 35 mL of 1X PBS).
2. Place the lids on the Sample and Elution Vessels, making sure that the vessels are sealed all the way around.
3. Ensure the Pathatrix® paramagnetic beads (included in the kit) are fully resuspended by agitating the bead vial (for example, by vortexing bead vial or inversion of sealed vial), and add 50 µL of the Pathatrix® paramagnetic bead suspension into the spout on the lid of the Sample Vessel.



4. Remove the capture-phase kit from the bag and orient with the valve plunger pointing left. Connect the valve firmly to the lids of the Sample and Elution Vessels.



5. Holding both vessels, lift the assembled vessels and attached capture-phase kit out of the Sample Vessel Holder.
6. Place the vessels into the Cartridge, pushing them in firmly from the bottom upwards.
7. Firmly push the rest of the kit into the Cartridge, ensuring that the valve, the capture phase, and the syringe are all held securely in the molded recess of the Cartridge.
8. Push the magnet slider across into the locking position and press the magnet release button on the side of the Cartridge to ensure the kit is positioned correctly and the magnets can freely disengage from the capture phase.



9. Reset the magnets into the locking position.
10. Insert the Cartridge into the Pathatrix® Auto Instrument until it clicks into the locking position.
11. Press the numbered button above the appropriate Cartridge to start the run (approximately 14 minutes). The associated LED will turn green to indicate the run has started.

Sample unloading

1. At the end of the run, the LED will flash red and green alternately.
2. Press the button above the appropriate Cartridge to initialize the draining step (approximately 1 minute).
3. When the draining step is complete and the LED is illuminated red, remove the Cartridge by pulling it out, away from the instrument.
4. Remove the syringe from the Cartridge and carefully pull out the rest of the kit, starting at the top and working downwards. When removing the Sample and Elution Vessels from the Cartridge, hold firmly by the vessels themselves to prevent spillage.
5. Place both vessels in the Tube Rack (also known as the Sample Vessel Holder or rack).
6. Remove the lid from the Elution Vessel and, leaving the Elution Vessel in place in the rack, lift away the rest of the consumable, including the Sample Vessel, and discard.
7. Place the Elution Vessel in the rack and cap with a flat lid (provided in the kit). Leave in the rack for 1 minute to allow capture of the Pathatrix® paramagnetic beads.
8. Remove all the liquid from the Elution Vessel, without removing the vessel from the rack, taking care not to disturb the captured Pathatrix® paramagnetic beads.
9. Remove the Elution Vessel from the vessel holder, add 100 µL of PBS into the Elution Vessel, and resuspend the Pathatrix® paramagnetic beads.
10. Appropriate aliquots of the Pathatrix® paramagnetic bead suspension can then be immediately analyzed using the laboratory's chosen pathogen detection method.

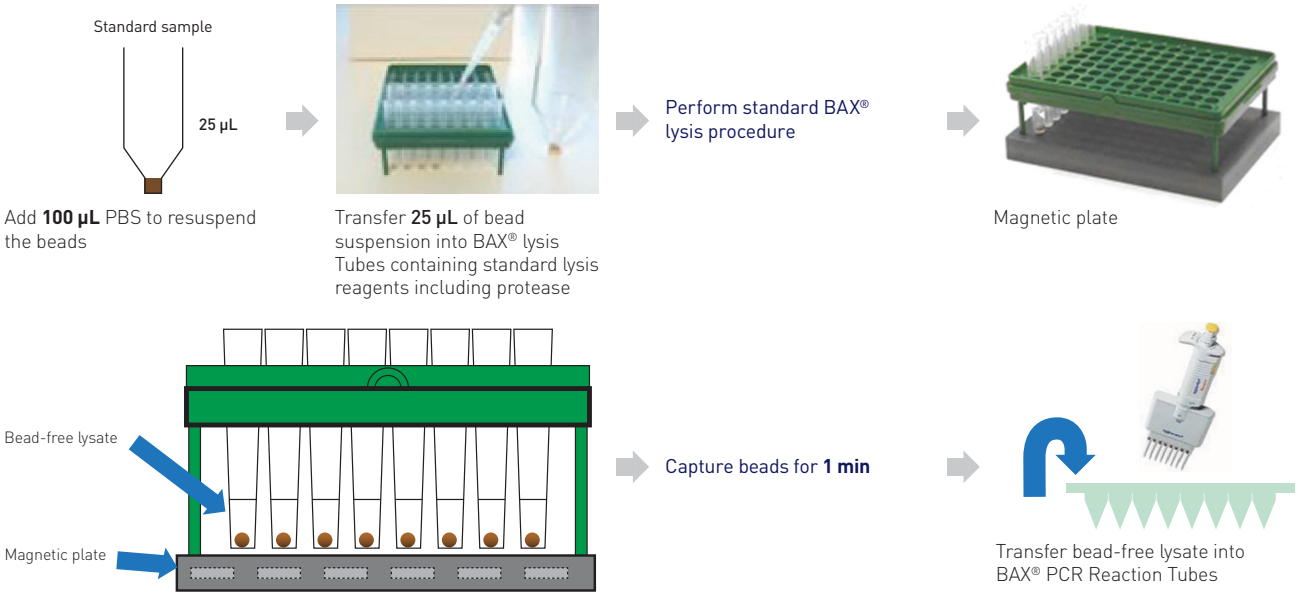
Note: The Pathatrix® paramagnetic bead suspension may be retained for later testing if necessary. The beads should be stored in 1.5-mL microcentrifuge tubes AWAY from magnets (for example, away from Pathatrix® vessel holders) at 5 ±3°C for up to 24 hours.

Detection – BAX® PCR protocol

IMPORTANT! It is solely the operator's responsibility to refer to the package insert instructions and instrument user manual for information regarding the correct set up and operation of the BAX® PCR system.

1. Pipet 25 µL of the Pathatrix® paramagnetic bead suspension into BAX® lysis tubes and proceed according to standard BAX® lysis procedure.
2. Once the lysis step is complete, immediately place the Rack and Tubes onto the Magnetic Capture Plate (Cat. no. MAGNETICPLATE) and leave for at least 1 minute to allow the Pathatrix® paramagnetic beads to accumulate on the bottom of the tube.
3. Transfer only **bead-free lysate** into the BAX® PCR reaction tubes

   Note: Target DNA, if present, will be in the **bead-free** supernatant.
4. To proceed, refer to the DuPont® Qualicon operating instructions.



If a negative result is obtained from the pooled sample, the individual overnight enrichments can be discarded, as further testing is not required.

If a positive result is obtained from the pooled sample, the overnight enrichments can be retested to allow identification of which individual samples in the pool produced the positive result (see the following section, "Retesting of individual samples after "Positive Pooled Sample"").

Retesting of individual samples after "Positive Pooled Sample"

1. Individual samples, which require retesting, **should be rewarmed to 37 ±1°C** before having **10 mL** removed and transferred into a Sample Vessel.
2. Individual samples should be retested immediately by repeating all the steps in Samples and consumable loading through Detection – BAX® PCR protocol described above.

If a positive PCR result is subsequently obtained, an aliquot of the Pathatrix® paramagnetic beads should be plated out (see the following section, "Detection – direct plating (optional)").

Detection – direct plating (optional)

1. Streak 10 µL of the remaining unlysed Pathatrix® paramagnetic bead suspension onto selective agar plates (for example, Cefixime Potassium Tellurite Sorbitol-MacConkey Agar [CT-SMAC], CHROMagar O157) for isolation of the target.

   Note: We recommend streaking the sample out to generate individual colonies, as opposed to spread plating.
2. Allow the plates to dry for approximately 10 minutes then invert and incubate at at the required temperature for 18–24 hours.

A presumptive positive result is defined as the isolation of typical, suspicious, or atypical *E. coli* O157:H7 colonies on the agar plates used. A presumptive positive isolate should be subsequently confirmed by the use of subculture, appropriate biochemical and serological tests (for example, as detailed in ISO 16654:2001 or USDA Microbiology Laboratory Guidebook [MLG] 5.04 as used in the AOAC Research Institute validation study [See "References" on page 53]).

Test result interpretation and classification

The Pathatrix® 5-Pooling *E. coli* O157:H7 Kit is designed as a sample preparation method for presence/absence detection of *E. coli* O157:H7 in food matrices

Using the Pathatrix® 5-Pooling *E. coli* O157:H7 Kit (overnight-enrichment format) linked to the BAX® PCR system, presumptive results can be obtained, prior to confirmation, within 20–23 hours.

Once confirmed, the results are reported as:

- *E. coli* O157:H7 **Detected** in 25–375 g (sample matrices)
- *E. coli* O157:H7 **Not detected** in 25–375 g (sample matrices)

3 Pathatrix® Kit Linked to the Idaho Technology R.A.P.I.D.® LT PCR System – Same-Day–Enrichment Format

Workflow

Sample enrichment (page 28)

▼

Sample preparation (page 29)

▼

Samples and consumable loading (page 30)

▼

Sample unloading (page 32)

▼

Detection – R.A.P.I.D.® LT PCR protocol (page 33)

▼

Retesting of individual samples after "Positive Pooled Sample" (page 34)

▼

Detection – direct plating (optional) (page 34)

▼

Test result interpretation and classification (page 35)

Procedural guidelines

- Use aseptic technique and good laboratory practices at all times.
- A facemask should be worn when weighing out powders.
- Care must be taken when boiling agar prior to autoclaving, and heat-resistant gloves should be worn when handling hot flasks of liquid.
- Take care when handling plates or vessels that contain microorganisms.
- Avoid generating aerosols, as pathogenic organisms may be present.
- Used or unused reagents, used media, sample enrichments, as well as other contaminated disposable materials should be disposed of following procedures for infectious or potentially infectious products.
- Sample enrichments might be contaminated with pathogenic organisms infectious to humans, so all waste must be treated as biohazardous and handled and disposed using safe laboratory practices, in accordance and compliance with all appropriate regulations.

Sample enrichment

1. Weigh the food sample (typically 25–375 g) into an appropriate sterile bag.
   - For sample sizes between 25–55 g, prepare a 1:10 dilution of the food sample in **pre-warmed (42 ±1**°C**)** Buffered Peptone Water. For example, add 25 g of food sample to 225 mL of prewarmed media.
   - Sample sizes above 55 g should be diluted in 500 mL of pre-warmed Buffered Peptone Water.

   IMPORTANT! It is critical that the enrichment media is prewarmed to 42 ±1°C prior to adding the food sample. To prevent cooling, all samples should then immediately be placed in the incubator at 42 ±1°C.

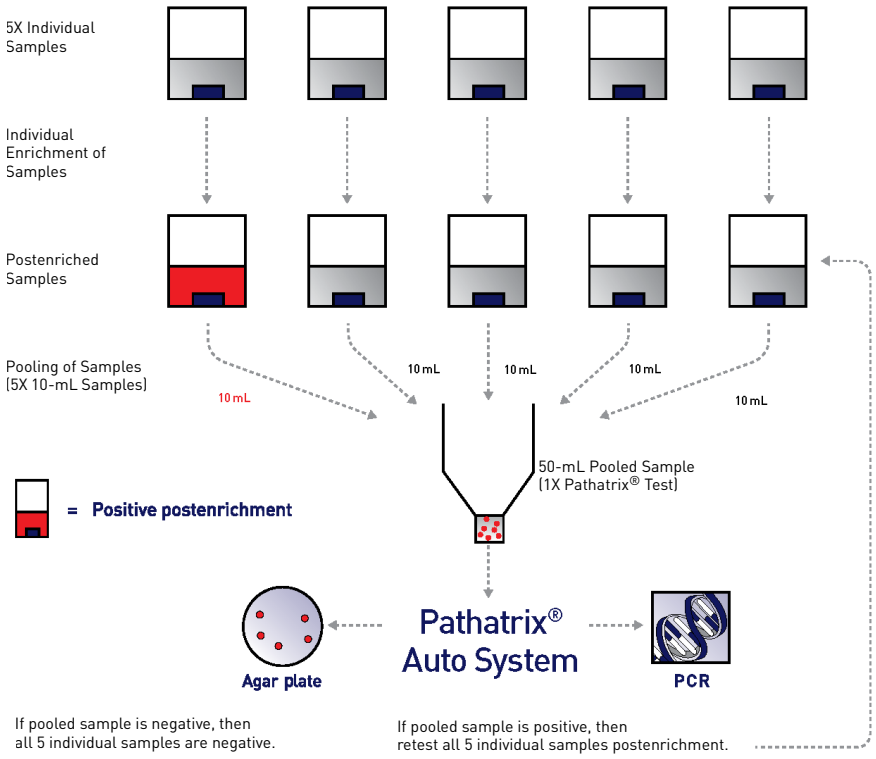
2. Homogenize the sample by massaging the sample between the fingers for 10–15 seconds to disperse any large clumps of material (Narang and Cray, 2006).
3. Incubate at **42 ±1**°C for a **minimum** of **6 hours**.

   Note: We recommend that these sub-samples for pooling are processed immediately or, if storage is required, that the samples are refrigerated at 5 ±3°C. Samples should be rewarmed to 37 ±1°C prior to analysis on the Pathatrix® Auto Instrument. The remaining enriched sample should be stored at 5 ±3°C for up to 32 hours until the results of the pooled sample have been determined.

Sample preparation

1. Remove the Sample and Elution Vessels from the consumable kit packaging and place into the Sample Vessel Holder.
2. Partially remove the lids from both vessels, making a large enough opening to allow the sample and wash buffer to be dispensed into the vessels.
3. Prepare a single, pooled sample in the Sample Vessel by pooling **5 × 10-mL** aliquots from 5 individually enriched samples to create a 50-mL pooled sample (see figure below).

   Note: If the samples are highly particulate and/or contain a high fat content, the use of the FiltaFoam system (Foam filters, Cat. no. PFF) with pooling syringes and straws (Cat. no. POOL510MLN) is recommended. Alternatively, Seward plain sterile bags with internal strainers may be used (Seward Product Code BA6041/STR).

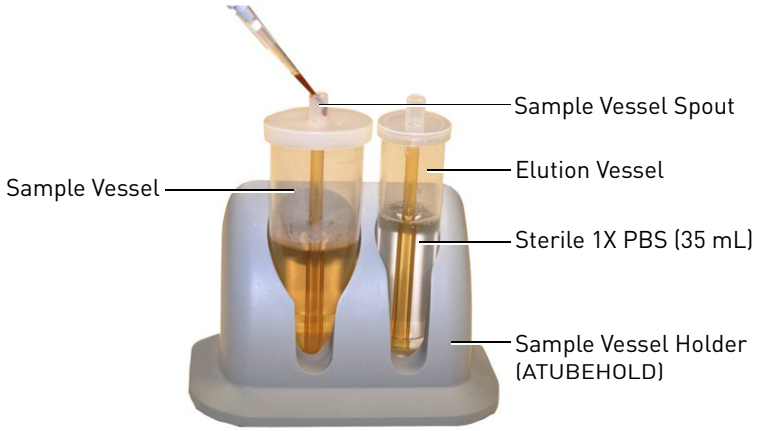


4. Store the individual enriched samples at 5 ±3°C for potential reanalysis until the test result is confirmed.

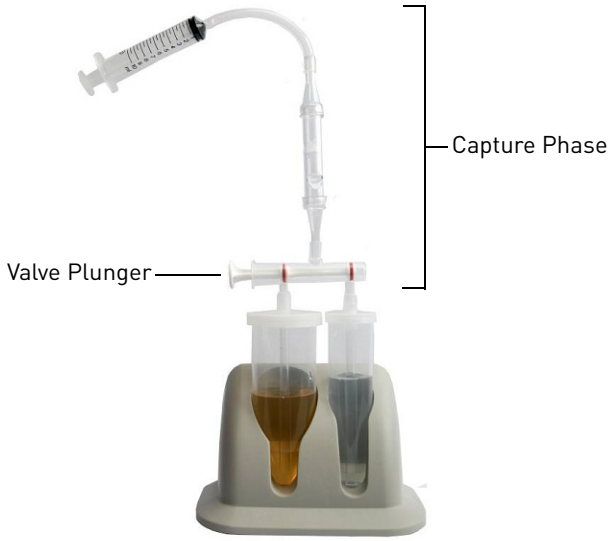
   Note: Do not store for more than 32 hours.

Samples and consumable loading

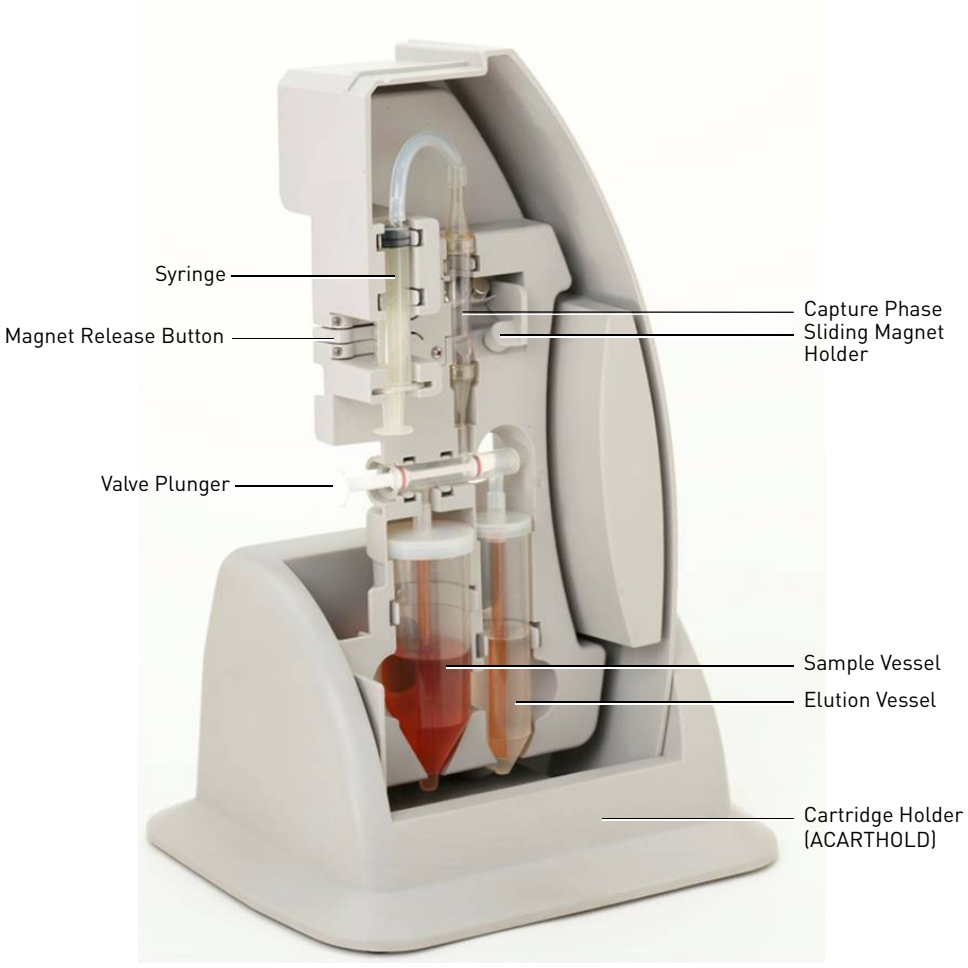
1. Add PBS (Cat. no. AM9624, diluted to 1X) to the fill line of the Elution Vessel (approximately 35 mL of 1X PBS).
2. Place the lids on the Sample and Elution Vessels, making sure that the vessels are sealed all the way around.
3. Ensure the Pathatrix® paramagnetic beads (included in the kit) are fully resuspended by agitating the bead vial (for example, by vortexing bead vial or inversion of sealed vial), and add 50 µL of the Pathatrix® paramagnetic bead suspension into the spout on the lid of the Sample Vessel.



4. Remove the capture-phase kit from the bag and orientate with the valve plunger pointing left. Connect the valve firmly to the lids of the Sample and Elution Vessels.



5. Holding both vessels, lift the assembled vessels and attached capture-phase kit out of the Sample Vessel Holder.
6. Place the vessels into the Cartridge, pushing them in firmly from the bottom upwards.
7. Firmly push the remainder of the kit into the Cartridge, ensuring that the valve, the capture phase, and the syringe are all held securely in the molded recess of the Cartridge.
8. Push the magnet slider across into the locking position and press the magnet release button on the side of the Cartridge to ensure the kit is positioned correctly and the magnets can freely disengage from the capture phase.



9. Reset the magnets into the locking position.
10. Insert the Cartridge into the Pathatrix® Auto Instrument until it clicks into the locking position.
11. Press the numbered button above the appropriate Cartridge to start the run (approximately 14 minutes). The associated LED will turn green to indicate the run has started.

Sample unloading

1. At the end of the run, the LED will flash red and green alternately.
2. Press the button above the appropriate Cartridge to initialize the draining step (approximately 1 minute).
3. When the draining step is complete and the LED is illuminated red, remove the Cartridge by pulling it out, away from the instrument.
4. Remove the syringe from the Cartridge and carefully pull out the rest of the kit, starting at the top and working downwards. When removing the Sample and Elution Vessels from the Cartridge, hold firmly by the vessels themselves to prevent spillage.
5. Place both vessels in the Tube Rack (also known as the Sample Vessel Holder or rack).
6. Remove the lid from the Elution Vessel and, leaving the Elution Vessel in place in the rack, lift away the rest of the consumable, including the Sample Vessel, and discard.
7. Place the Elution Vessel in the rack and cap with a flat lid (provided in the kit). Leave in the rack for 1 minute to allow capture of the Pathatrix® paramagnetic beads.
8. Remove all the liquid from the Elution Vessel, without removing the vessel from the rack, taking care not to disturb the captured Pathatrix® paramagnetic beads.
9. Remove the Elution Vessel from the vessel holder, add 100 µL of PBS into the Elution Vessel, and resuspend the Pathatrix® paramagnetic beads.
10. Appropriate aliquots of the Pathatrix® paramagnetic bead suspension can then be immediately analyzed using the laboratory's chosen pathogen detection method.

Note: The Pathatrix® paramagnetic bead suspension may be retained for later testing if necessary. The beads should be stored in 1.5-mL microcentrifuge tubes AWAY from magnets (for example, away from Pathatrix® vessel holders) at 5 ±3°C for up to 24 hours.

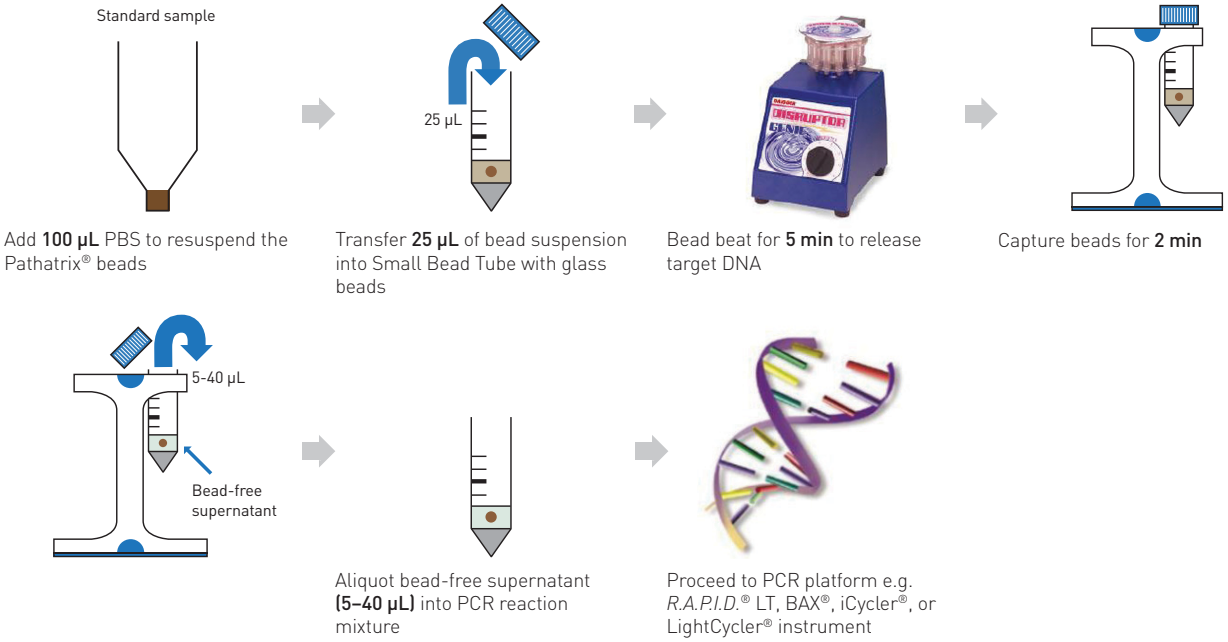
Detection – R.A.P.I.D.® LT PCR protocol

IMPORTANT! It is solely the operator's responsibility to refer to the package insert instructions and instrument user manual for information regarding the correct set up and operation of the R.A.P.I.D.® LT PCR system.

1. Add 25 µL of the resuspended Pathatrix® paramagnetic beads into a "Blue Capped Bead Tube" and vortex on the Disruptor Genie® vortexer (equipped with the TurboMix™ attachment) for 5 minutes on the highest setting.
2. Once the bead-beating lysis step is complete, immediately place the Bead Tubes into the DynaMag™-2 Magnet (Cat. no. 123.21D).
3. Wait for at least 1 minute to allow the Pathatrix® paramagnetic bead debris to be drawn out of suspension, thereby producing the **bead-free** supernatant.

   Note: If present, target DNA will be in the bead-free lysate.
4. Pipet 10 µL of Reconstitution Buffer into the "Unknown" test vial.
5. Pipet 10 µL of bead-free supernatant into the PCR "Unknown" test reaction vial. Mix well to fully resuspend the PCR reagent pellet.

   Note: Take care not to transfer any glass beads from the Blue Capped Bead-Beating Tubes into the PCR reagent vial.
6. Transfer 18 µL of the reagent/sample suspension, and add into the top of a glass PCR capillary tube. Using the "Capping Tool" provided, place the cap on the sample capillary tube and push down firmly.
7. To proceed, refer to the R.A.P.I.D.® LT PCR operating instructions.



Review the amplification curves and melt peaks as described in the R.A.P.I.D.® LT PCR software "Screen Shot Protocol." If any Amplification Curve exhibits an increase in fluorescence and/or displays a potential Melt Peak in the absence of a positive PCR software determination, a repeat PCR analysis should be carried out by repeating the R.A.P.I.D.® LT PCR steps in this section (see page 33).

If a negative result is obtained from the pooled sample, the individual enrichments can be discarded, as further testing is not required.

If a positive result is obtained from the pooled sample, the individual enrichments can be retested to allow identification of which individual samples in the pool produced the positive result (see the following section, "Retesting of individual samples after "Positive Pooled Sample"").

Retesting of individual samples after "Positive Pooled Sample"

1. Individual samples, which require retesting, **should be rewarmed to 42 ±1°C** before having **10 mL** removed and transferred into a Sample Vessel.
2. Individual samples should be retested immediately by repeating all the steps in Samples and consumable loading through Detection – R.A.P.I.D.® LT PCR protocol described above.

If a positive PCR result is subsequently obtained, an aliquot of the Pathatrix® paramagnetic beads should be plated out (see the following section, "Detection – direct plating (optional)").

Detection – direct plating (optional)

Note: We recommend streaking the sample out to generate individual colonies, as opposed to spread plating.

1. Pipet all of the remaining Pathatrix® paramagnetic bead suspension onto the edge of well-dried selective agar plates (for example, Cefixime Potassium Tellurite Sorbitol-MacConkey Agar [CT-SMAC], CHROMagar O157).

   Note: Retain an amount of bead suspension you wish to keep. Divide the remainder of the bead suspension into equal amounts for streaking on your selective agar plates.
2. Using a sterile 10-µL inoculation loop, streak from this pool to generate isolated colonies.
3. Allow the plates to dry for approximately 10 minutes then invert and incubate at at the required temperature for 18–24 hours.

A presumptive positive result is defined as the isolation of typical, suspicious, or atypical *E. coli* O157:H7 colonies on the agar plates used. A presumptive positive isolate should be subsequently confirmed by the use of subculture and appropriate biochemical and serological tests (for example, as detailed in ISO 16654:2001 or USDA Microbiology Laboratory Guidebook [MLG] 5.04 as used in the AOAC Research Institute validation study [See "References" on page 53]).

Test result interpretation and classification

The Pathatrix® 5-Pooling *E. coli* O157:H7 Kit is designed as a sample preparation method for presence/absence detection of *E. coli* O157:H7 in food matrices

Using the Pathatrix® 5-Pooling *E. coli* O157:H7 Kit (same-day–enrichment format) linked to the R.A.P.I.D.® LT PCR system, presumptive results can be obtained, prior to confirmation, within 7–9 hours.

Once confirmed, the results are reported as:

- *E. coli* O157:H7 **Detected** in 25–375 g (sample matrices)
- *E. coli* O157:H7 **Not detected** in 25–375 g (sample matrices)

4 Pathatrix® Kit Linked to the Idaho Technology R.A.P.I.D.® LT PCR System – Overnight-Enrichment Format

Workflow

Sample enrichment (page 38)

▼

Sample preparation (page 39)

▼

Samples and consumable loading (page 40)

▼

Sample unloading (page 42)

▼

Detection – R.A.P.I.D.® LT PCR protocol (page 43)

▼

Retesting of individual samples after "Positive Pooled Sample" (page 44)

▼

Detection – direct plating (optional) (page 44)

▼

Test result interpretation and classification (page 45)

Procedural guidelines

- Use aseptic technique and good laboratory practices at all times.
- A facemask should be worn when weighing out powders.
- Care must be taken when boiling agar prior to autoclaving, and heat-resistant gloves should be worn when handling hot flasks of liquid.
- Take care when handling plates or vessels that contain microorganisms.
- Avoid generating aerosols, as pathogenic organisms may be present.
- Used or unused reagents, used media, sample enrichments, as well as other contaminated disposable materials should be disposed of following procedures for infectious or potentially infectious products.
- Sample enrichments might be contaminated with pathogenic organisms infectious to humans, so all waste must be treated as biohazardous and handled and disposed using safe laboratory practices, in accordance and compliance with all appropriate regulations.

Sample enrichment

1. Weigh the food sample (typically 25–375 g) into an appropriate sterile bag.
   - For sample sizes between 25–55 g, prepare a 1:10 dilution of the food sample in **pre-warmed (42 ±1°C)** Buffered Peptone Water. For example, add 25 g of food sample to 225 mL of prewarmed media.
   - Sample sizes above 55 g should be diluted in 500 mL of pre-warmed Buffered Peptone Water.

   IMPORTANT! It is critical that the enrichment media is prewarmed to 42 ±1°C prior to adding the food sample. To prevent cooling, all samples should then immediately be placed in the incubator at 42 ±1°C.

   ---

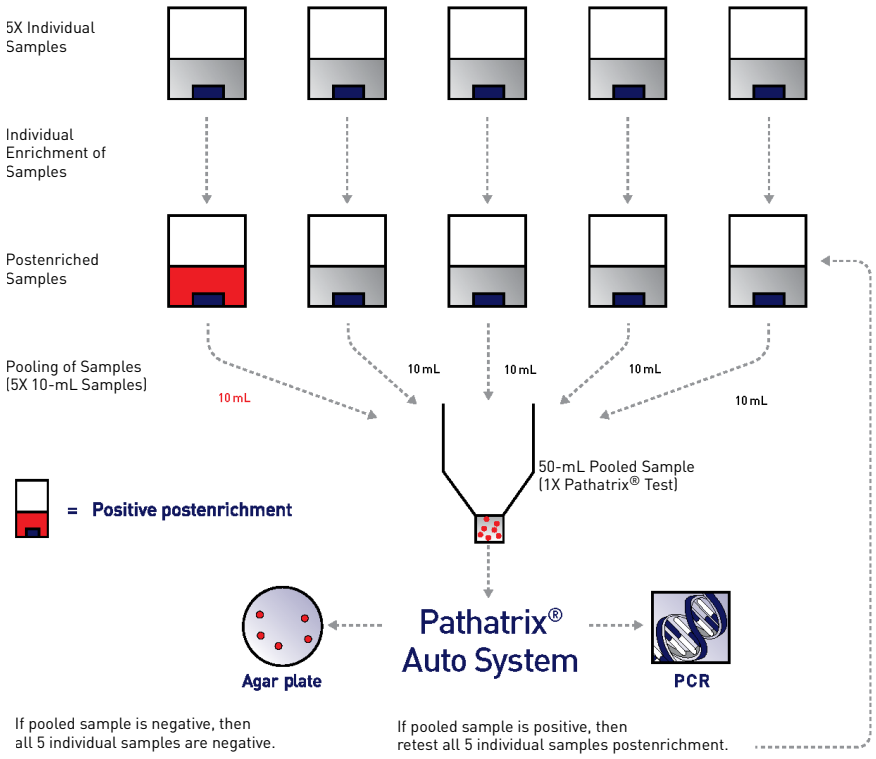
2. Homogenize the sample by massaging the sample between the fingers for 10–15 seconds to disperse any large clumps of material (Narang and Cray, 2006).
3. Incubate at **42 ±1**°C for a **minimum** of **16 hours** up to a **maximum** of **24 hours**.

   Note: We recommend that these sub-samples for pooling are processed immediately or, if storage is required, that the samples are refrigerated at 5 ±3°C. Samples should be rewarmed to 37 ±1°C prior to analysis on the Pathatrix® Auto Instrument. The remaining enriched sample should be stored at 5 ±3°C for up to 32 hours until the results of the pooled sample have been determined.

Sample preparation

1. Remove the Sample and Elution Vessels from the consumable kit packaging and place into the Sample Vessel Holder.
2. Partially remove the lids from both vessels, making a large enough opening to allow the sample and wash buffer to be dispensed into the vessels.
3. Prepare a single, pooled sample in the Sample Vessel by pooling **5 × 10-mL** aliquots from 5 individually enriched samples to create a 50-mL pooled sample (see figure below).

   Note: If the samples are highly particulate and/or contain a high fat content, the use of the FiltaFoam system (Foam filters, Cat. no. PFF) with pooling syringes and straws (Cat. no. POOL510MLN) is recommended. Alternatively, Seward plain sterile bags with internal strainers may be used (Seward Product Code BA6041/STR).

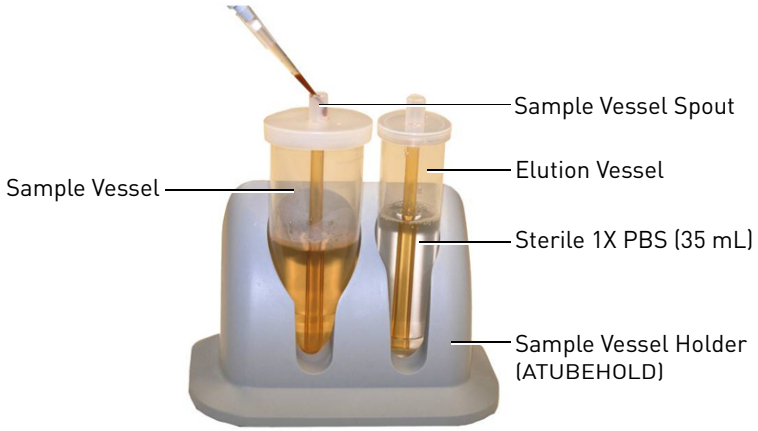


4. Store the individual enriched samples at 5 ±3°C for potential reanalysis until the test result is confirmed.

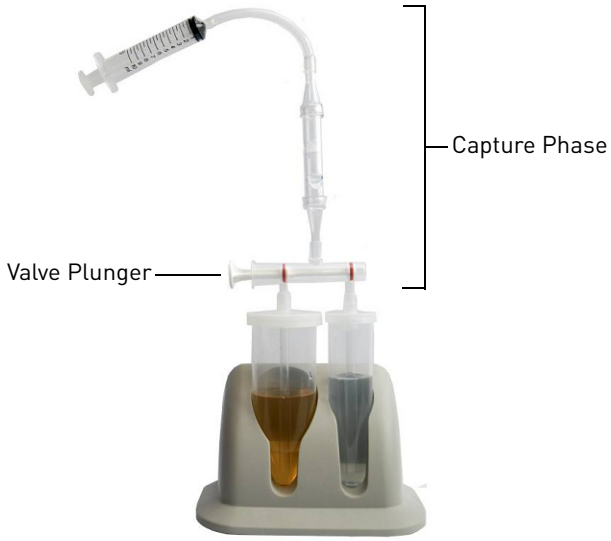
   Note: Do not store for more than 32 hours.

Samples and consumable loading

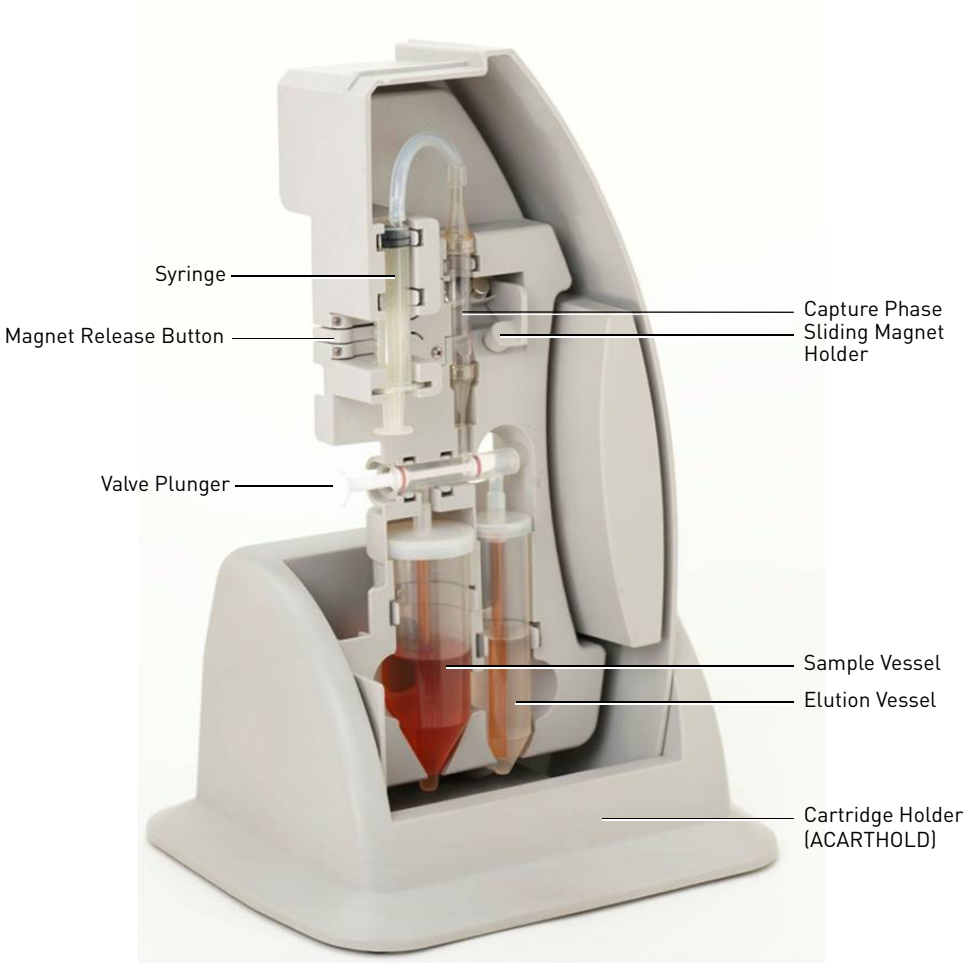
1. Add PBS (Cat. no. AM9624, diluted to 1X) to the fill line of the Elution Vessel (approximately 35 mL of 1X PBS).
2. Place the lids on the Sample and Elution Vessels, making sure that the vessels are sealed all the way around.
3. Ensure the Pathatrix® paramagnetic beads (included in the kit) are fully resuspended by agitating the bead vial (for example, by vortexing bead vial or inversion of sealed vial), and add 50 µL of the Pathatrix® paramagnetic bead suspension into the spout on the lid of the Sample Vessel.



4. Remove the capture-phase kit from the bag and orient with the valve plunger pointing left. Connect the valve firmly to the lids of the Sample and Elution Vessels.



5. Holding both vessels, lift the assembled vessels and attached capture-phase kit out of the Sample Vessel Holder.
6. Place the vessels into the Cartridge, pushing them in firmly from the bottom upwards.
7. Firmly push the remainder of the kit into the Cartridge, ensuring that the valve, the capture phase, and the syringe are all held securely in the molded recess of the Cartridge.
8. Push the magnet slider across into the locking position and press the magnet release button on the side of the Cartridge to ensure the kit is positioned correctly and the magnets can freely disengage from the capture phase.



9. Reset the magnets into the locking position.
10. Insert the Cartridge into the Pathatrix® Auto Instrument until it clicks into the locking position.
11. Press the numbered button above the appropriate Cartridge to start the run (approximately 14 minute). The associated LED will turn green to indicate the run has started.

Sample unloading

1. At the end of the run, the LED will flash red and green alternately.
2. Press the button above the appropriate Cartridge to initialize the draining step (approximately 1 minute).
3. When the draining step is complete and the LED is illuminated red, remove the Cartridge by pulling it out, away from the instrument.
4. Remove the syringe from the Cartridge and carefully pull out the rest of the kit, starting at the top and working downwards. When removing the Sample and Elution Vessels from the Cartridge, hold firmly by the vessels themselves to prevent spillage.
5. Place both vessels in the Tube Rack (also known as the Sample Vessel Holder or rack).
6. Remove the lid from the Elution Vessel and, leaving the Elution Vessel in place in the rack, lift away the rest of the consumable, including the Sample Vessel, and discard.
7. Place the Elution Vessel in the rack and cap with a flat lid (provided in the kit). Leave in the rack for 1 minute to allow capture of the Pathatrix® paramagnetic beads.
8. Remove all the liquid from the Elution Vessel, without removing the vessel from the rack, taking care not to disturb the captured Pathatrix® paramagnetic beads.
9. Remove the Elution Vessel from the vessel holder, add 100 µL of PBS into the Elution Vessel, and resuspend the Pathatrix® paramagnetic beads.
10. Appropriate aliquots of the Pathatrix® paramagnetic bead suspension can then be immediately analyzed using the laboratory's chosen pathogen detection method.

Note: The Pathatrix® paramagnetic bead suspension may be retained for later testing if necessary. The beads should be stored in 1.5-mL microcentrifuge tubes AWAY from magnets (for example, away from Pathatrix® vessel holders) at 5 ±3°C for up to 24 hours.

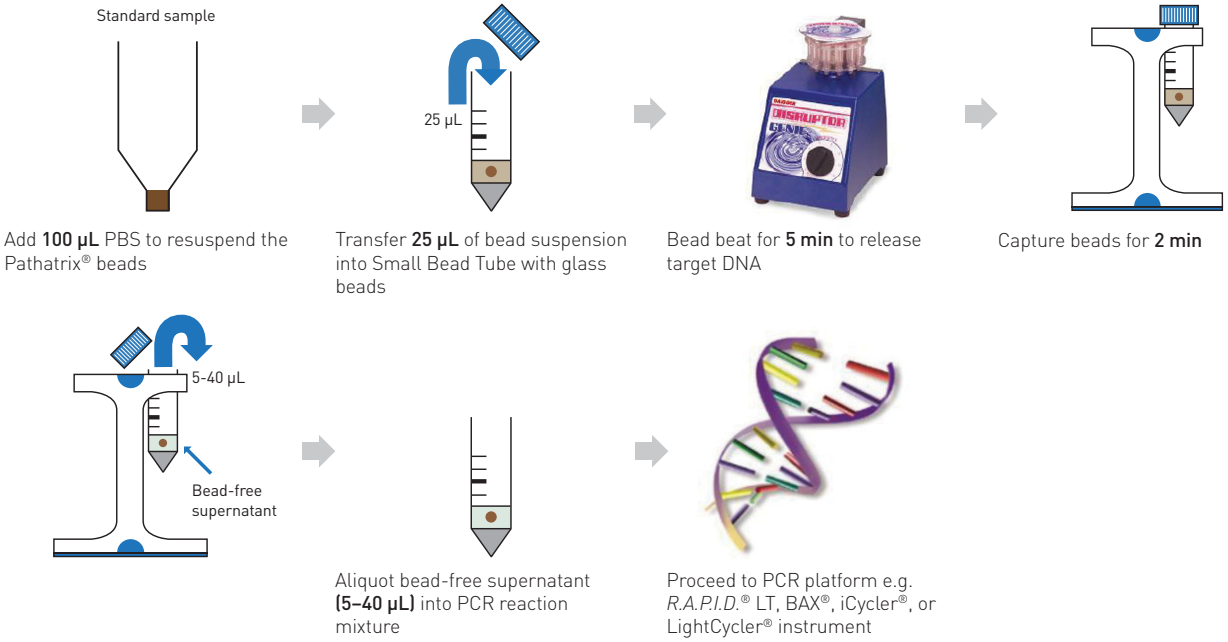
Detection – R.A.P.I.D.® LT PCR protocol

IMPORTANT! It is solely the operator's responsibility to refer to the package insert instructions and instrument user manual for information regarding the correct set up and operation of the R.A.P.I.D.® LT PCR system.

1. Add 25 µL of the resuspended Pathatrix® paramagnetic beads into a "Blue Capped Bead Tube" and vortex on the Disruptor Genie® vortexer (equipped with the TurboMix™ attachment) for 5 minutes on the highest setting.
2. Once the bead-beating lysis step is complete, immediately place the Bead Tubes into the DynaMag™-2 Magnet (Cat. no. 123.21D).
3. Wait for at least 1 minute to allow the Pathatrix® paramagnetic bead debris to be drawn out of suspension, thereby producing the **bead-free** supernatant.

   Note: If present, target DNA will be in the bead-free lysate.
4. Pipet 10 µL of Reconstitution Buffer into the "Unknown" test vial.
5. Pipet 10 µL of bead-free supernatant into the PCR "Unknown" test reaction vial. Mix well to fully resuspend the PCR reagent pellet.

   Note: Take care not to transfer any glass beads from the Blue Capped Bead-Beating Tubes into the PCR reagent vial.
6. Transfer 18 µL of the reagent/sample suspension, and add into the top of a glass PCR capillary tube. Using the "Capping Tool" provided, place the cap on the sample capillary tube and push down firmly.
7. To proceed, refer to the R.A.P.I.D.® LT PCR operating instructions.



Review the amplification curves and melt peaks as described in the R.A.P.I.D.® LT PCR software "Screen Shot Protocol." If any Amplification Curve exhibits an increase in fluorescence and/or displays a potential Melt Peak in the absence of a positive PCR software determination, a repeat PCR analysis should be carried out by repeating the R.A.P.I.D.® LT PCR steps in this section (see page 43).

If a negative result is obtained from the pooled sample, the individual overnight enrichments can be discarded, as further testing is not required.

If a positive result is obtained from the pooled sample, the individual enrichments can be retested to allow identification of which individual samples in the pool produced the positive result (see the following section, "Retesting of individual samples after "Positive Pooled Sample"").

Retesting of individual samples after "Positive Pooled Sample"

1. Individual samples that require retesting **should be rewarmed to 42 ±1°C** before having **10 mL** removed and transferred into a Sample Vessel.
2. Individual samples should be retested immediately by repeating all the steps in Samples and consumable loading through Detection – R.A.P.I.D.® LT PCR protocol described above.

If a positive PCR result is subsequently obtained, an aliquot of the Pathatrix® paramagnetic beads should be plated out (see the following section, "Detection – direct plating (optional)").

Detection – direct plating (optional)

Note: We recommend streaking the sample out to generate individual colonies, as opposed to spread plating.

1. Streak 10 µL of the remaining Pathatrix® paramagnetic bead suspension onto well-dried selective agar plates (for example, Cefixime Potassium Tellurite Sorbitol-MacConkey Agar [CT-SMAC], CHROMagar O157) for isolation of the target.
2. Allow the plates to dry for approximately 10 minutes then invert and incubate at at the required temperature for 18–24 hours.

A presumptive positive result is defined as the isolation of typical, suspicious, or atypical *E. coli* O157:H7 colonies on the agar plates used. A presumptive positive isolate should be subsequently confirmed by the use of subculture and appropriate biochemical and serological tests (for example, as detailed in ISO 16654:2001 or USDA Microbiology Laboratory Guidebook [MLG] 5.04 as used in the AOAC Research Institute validation study [See "References" on page 53]).

Test result interpretation and classification

The Pathatrix® 5-Pooling *E. coli* O157:H7 Kit is designed as a sample preparation method for presence/absence detection of *E. coli* O157:H7 in food matrices

Using the Pathatrix® 5-Pooling *E. coli* O157:H7 Kit (overnight-enrichment format) linked to the R.A.P.I.D.® LT PCR system, presumptive results can be obtained, prior to confirmation, within 18–21 hours.

Once confirmed, the results are reported as:

- *E. coli* O157:H7 **Detected** in 25–375 g (sample matrices)
- *E. coli* O157:H7 **Not detected** in 25–375 g (sample matrices)

A

Background

Product overview

Description of target microorganisms

Escherichia coli O157:H7 is a major foodborne pathogen that causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans. *E. coli* O157:H7 is one of the main enterohemorrhagic *E. coli* serotypes which secrete Shiga-like toxins. Outbreaks of *E. coli* O157:H7 have been associated with contaminated food supplies such as raw ground beef, spinach, unpasteurized juices, and water.

Audience

The Pathatrix® 5-Pooling *E. coli* O157:H7 Kit is for professional use only and is intended for use by qualified users interested in determining the presence/absence of *E. coli* O157:H7 in food samples. Users may include, but are not limited to, food producers, food processors, food manufacturers, retailers, and microbiology testing laboratories.

Sampling protocol

The standard food sample size used in the Pathatrix® Auto system is 25 g of food diluted with 225 mL of enrichment medium. We recommend that sub-samples for pooling are processed immediately or, if storage is required, that the samples are refrigerated at 5 ±3°C. Samples should be rewarmed to 37 ±1°C prior to analysis with the Pathatrix® Auto system. The remaining enriched sample should be stored at 5 ±3°C for up to 32 hours until the results of the pooled sample have been determined.

Kit sensitivity

The sample preparation procedure allows you to detect as few as 1–10 cfu from 25–375 g of food samples after enrichment. The limitation of the Pathatrix® 5-Pooling *E. coli* O157:H7 Kit is in the ability of the target to reproduce in the enrichment medium, be captured by the magnet, and subsequently be detected by BAX® PCR or R.A.P.I.D® LT PCR or be isolated on selective agar plates.

CAUTION! The Pathatrix® kit has been evaluated on raw ground beef. Given the wide variety of products and manufacturing procedures, we recommend that you check that the composition of the matrices to be tested does not affect the reliability of the results.

A negative result does not guarantee the absence of target organism in the original sample and may be due to the inability of the organism to adequately reproduce to required levels in the enrichment medium (with subsequent outgrowth on selective agar plates) potentially due to, but not limited to, competitive microflora, sub-lethal injury, or matrix inhibition.

Operating conditions

The Pathatrix® Auto Instrument is for indoor use only and for altitudes not exceeding 2000 m (6500 ft) above sea level.

Temperature and humidity requirements	
Condition	**Acceptable range**
Temperature	5–40°C
Humidity	Maximum relative humidity 80% for temperatures up to 31°C, decreasing to 50%

B

Ordering Information

Related materials from Life Technologies

Item	Cat. no.
Related consumable kits with associated beads	
Pathatrix® *E. coli* O157:H7 Kit	APE50
Pathatrix® 10-Pooling *E. coli* O157:H7 Kit	APE500SDP
Pathatrix® 5-Pool DUAL (*E. coli/Salmonella* spp.) Kit	APDES250P
Equipment	
Pathatrix® Auto Instrument	PATHATRIXAUTO
Cartridge Rack (optional for use with the Pathatrix® Auto Instrument; holds 5 Cartridges)	ACARTRACK
DynaMag™-2 Magnet (for use with microcentrifuge tubes)	123.21D
Reagents	
PBS, 10X, pH 7.4	AM9624 or AM9625
Consumables	
Foam filters	PFF
5-Pool Kit – Straws (254 mm) and Syringes (10 mL)	POOL510MLN
Related PCR assay	
MicroSEQ® *E. coli* O157:H7 Detection Kit	4427409 and 4445654 (with user guide and quick reference card)

C

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 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.

Specific chemical handling

CAS	Chemical	Phrase
26628-22-8	Sodium Azide	Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

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

Support email: **foodsafety@lifetech.com**

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)
- Search for user documents, SDSs, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

References

Food Safety and Inspection Service (USDA). 2008. Detection, isolation and identification of *Escherichia coli* O157:H7 from meat products. MLG 5.04. Microbiology Laboratory Guidebook.

ISO. 2001. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157. Reference number 16654:2001.

Narang, N. and Cray, W.C. 2006. Evaluation of Hand Mixing of Ground Beef and Poultry Samples as an Alternative to Stomaching for the Detection of *Salmonella*. *Food Protection Trends*. 26:14–19.

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