USER GUIDE

applied biosystems by life technologies™

Pathatrix® *Salmonella* spp. Kit (Individual Samples) Linked to Selective Agar Plates

For use with the Pathatrix® Auto Instrument

Catalog Number APS50

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PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 090203C

For testing of Food and Environmental samples only.

life technologies™

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Contents

Product Information

IMPORTANT! Before using this product, read and understand the information in Appendix D, "Safety" on page 20.

CAUTION! *Salmonella* spp. is a Biosafety Level 2 (BSL-2) organism [excluding *S. typhi* and *S. paratyphi*, which are both Biosafety Level 3 (BSL-3)]. Care must be taken when handling samples that may contain salmonellae. Laboratory personnel must be adequately trained to handle pathogens before being permitted to analyze samples for *Salmonella* spp. Laboratory personnel must wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. Waste should be disposed of in compliance with local and national legislation as appropriate.

About the kit

The Pathatrix® *Salmonella* spp. Kit provides a sample preparation method for presence/absence testing based on the detection of as few as 1–10 cfu (colony forming units)/25–325 g of food sample.

Using the Pathatrix® *Salmonella* spp. Kit (Individual Samples) Linked to Selective Agar Plates, presumptive results can be obtained, prior to confirmation, within 36 hours. 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:

- *Salmonella* spp. **Detected** in 25–325 g (sample matrices)
- *Salmonella* spp. **Not detected** in 25–325 g (sample matrices)

See Appendix B on page 17 for additional background information.

Kit contents

The Pathatrix® *Salmonella* spp. Kit (Cat. no. APS50) contains enough consumable components and Pathatrix® beads to process 50 samples.

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-*Salmonella* spp. 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® beads. Beads that have been subjected to freezing temperatures may be rendered inactive.

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

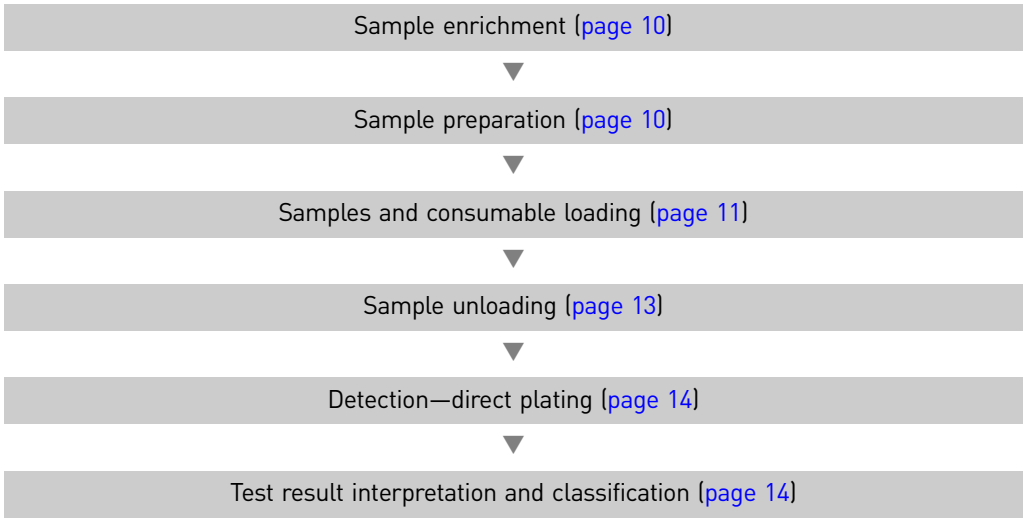
Materials not included in the kit

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).

Equipment, consumables, and reagents	
Item	**Source**
Equipment	
Incubator, 37 ±1°C	MLS
Pathatrix® Auto Instrument	Life Technologies Cat. no. PATHATRIXAUTO
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
Sterile, disposable 10-µL loops	MLS
Media	
See page 10 and Appendix A on page 15 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
For samples with high background microflora: Tetrathionate (TT) broth base	Oxoid product code CM0029
For milk powder, chocolate, or cocoa-based samples: • UHT skim milk • Brilliant green (CAS 633-03-4)	• Food retail store • MLS
Selective agar	
XLD agar	Oxoid product code CM0469
Brilliant green agar (modified)	Oxoid product code CM0329
Reagents	
PBS, 10X, pH 7.4	Life Technologies Cat. no. AM9624 or AM9625

Methods

Workflow



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

Note: Certain food types and swabs/sponges can benefit from an alternative enrichment strategy. (see Appendix A, "Alternative Enrichment Methods").

1. Prepare a 1 in 10 dilution of the food sample in the appropriate **prewarmed (37 ±1°C)** enrichment media in a sterile bag.

   Note: For example, add 25 g of food sample to 225 mL of prewarmed media or add 325 g of food sample to 2925 mL of prewarmed media.

   IMPORTANT! It is critical that the enrichment media is prewarmed to 37 ±1°C prior to adding the food sample. To prevent cooling, all samples should then immediately be placed in the incubator at 37 ±1°C.

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2. Homogenize the sample by hand mixing for 10–15 seconds. Massage the sample between the fingers to disperse any large clumps of material (Narang and Cray, 2006).

3. Incubate at **37 ±1°C** for a **minimum** of **16 hours**.

   Note: We recommend that sub-samples for analysis 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.

Sample preparation

1. Remove the Sample and Elution Vessels from the consumable kit packaging and place into the Tube Rack (also known as 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. Place 50 mL of your sample in the sample vessel.

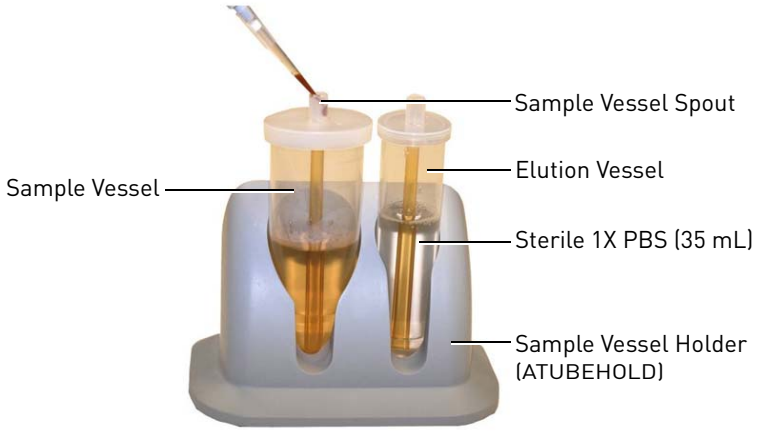
   Note: If your sample is highly particulate or has a high fat content, the use of the FiltaFoam system (Foam filters, Cat. no. PFF) with pooling syringes and straws (Cat. no. POOL510MLN) is recommended. Alternatively, the Seward plain sterile bag with internal strainer (Seward Product Code BA6041/STR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

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. If refrigerated after pre-enrichment, samples should be rewarmed to 37 ±1°C prior to removal of aliquots for analysis.

Samples and consumable loading

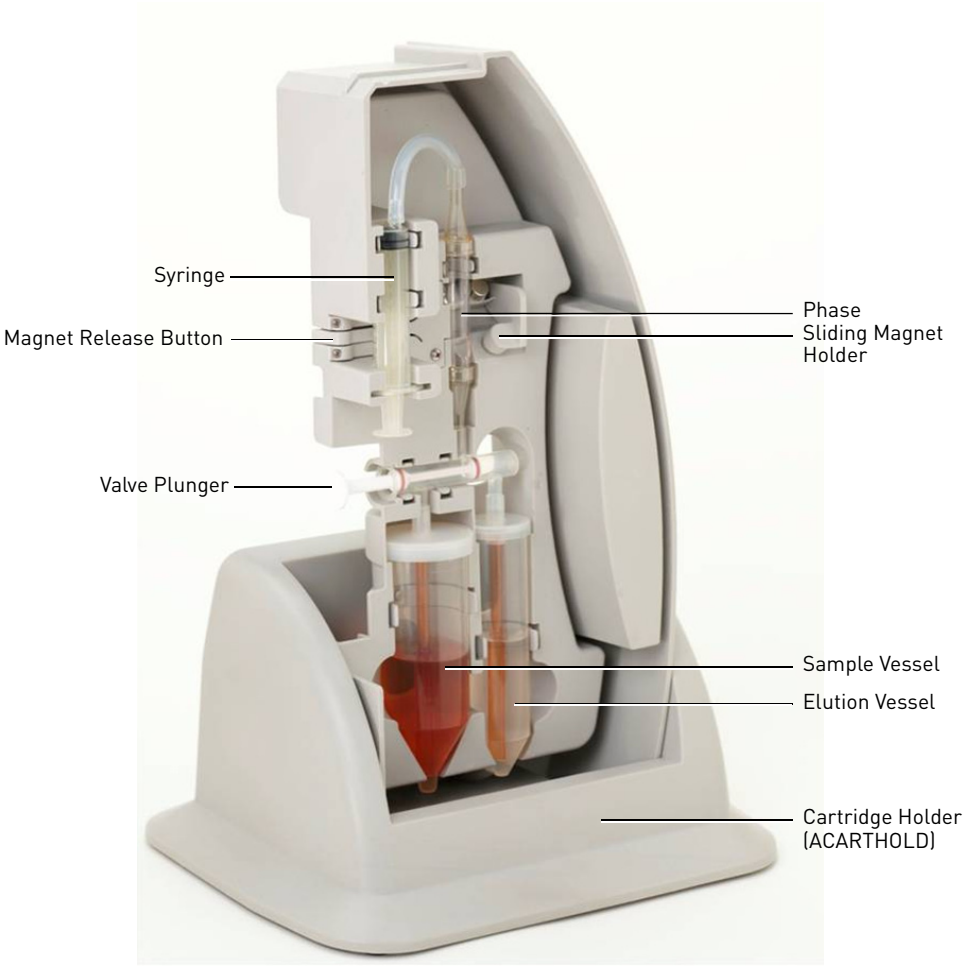
1. Add PBS (Cat. no. AM9624, diluted to 1X) to the fill line of the Elution Vessel (about 35 mL of 1X PBS).
2. Place the lids on to the Sample and Elution Vessels making sure that the vessels are sealed all the way around.
3. Ensure the beads are fully resuspended by agitating the bead vial (e.g., vortexing bead vial or inversion of sealed vial) and add 50 µL of the bead suspension (beads from Cat. no. APS50) into the spout on the lid of the Sample Vessel.



4. Remove the plastic 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 kit out of the rack (also known as the Sample Vessel Holder).

6. Place the vessels into the Cartridge, pushing them in firmly from the bottom upwards.
7. Next, firmly push the remainder of the kit into the Cartridge, ensuring that the valve, the 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 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. 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 a spare flat lid (provided in the consumable kit) on top of the Elution Vessel in the rack for 1 minute to allow capture of the beads.
8. Remove all the liquid from the Elution Vessel, without removing the vessel from the rack, taking care not to disturb the captured beads.
9. Add 100 µL of PBS into the Elution Vessel and resuspend the Pathatrix® beads.
10. Appropriate aliquots of the Pathatrix® bead suspension can then be immediately analyzed using the laboratory's chosen pathogen detection method.

Note: The bead suspension may be retained for later testing if necessary. The beads should be stored AWAY from magnets (e.g., Pathatrix® vessel holders) at 5 ±3°C for up to 24 hours.

Detection—direct plating

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

1. Streak 10 µL of the unlysed bead suspension over a well-dried XLD (xylose lysine desoxycholate) agar plate and another 10 µL onto an appropriate second selective plate medium (e.g., Brilliant Green Agar).

   Note: The laboratory may choose which medium to use, but the second selective plate should be any other solid selective medium complimentary to XLD and especially appropriate for the isolation of lactose-positive *Salmonella*, *Salmonella typhi*, and *Salmonella paratyphi* strains.

2. Allow the plates to dry for approximately 10 minutes then invert and incubate at at the required temperature for 18–24 hours or as recommended by the manufacturer.

A presumptive positive result is defined as the isolation of typical, suspicious, or atypical *Salmonella* spp. colonies on the agar plates used.

A presumptive positive isolate should be subsequently confirmed by the use of subculture, appropriate biochemical and serological tests as detailed in USDA Microbiology Laboratory Guidebook (MLG) 4.04 for cooked ham or FDA Bacteriological Analytical Manual (BAM), Chapter 5, sections D and E for tomatoes and chocolate (see "References" on page 23).

Test result interpretation and classification

The Pathatrix® *Salmonella* spp. Kit is designed as a sample preparation method for presence/absence detection of *Salmonella* spp. in food matrices.

Using the Pathatrix® *Salmonella* spp. Kit (Individual Samples) Linked to Selective Agar Plates, presumptive results can be obtained, prior to confirmation, within 36 hours.

Once confirmed, the results are reported as:

- *Salmonella* spp. **Detected** in 25–325 g (sample matrices)
- *Salmonella* spp. **Not detected** in 25–325 g (sample matrices)

A Alternative Enrichment Methods

Specific recommendations for high background microflora

For certain food groups, particularly where high levels of background microflora are present, the *Salmonella* assay results can be enhanced by the use of Tetrathionate (TT) broth instead of Buffered Peptone Water. The level of background contamination on the agar plates can be reduced by this method.

Examples of food groups that benefit from the use of TT broth are:

- Raw meat
- Raw vegetables
- Salads
- Fruits
- Ready meals

TT broth information

Materials

- Tetrathionate broth base is available from Oxoid (Product Code CM0029).
- Iodine Solution (to be added following preparation and heating of TT broth base).

  Note: Prepare the iodine solution in advance to allow the iodine to dissolve, but use on the same day that it is made.

Directions for use

1. Add 77 g of TT broth base to 1 L of distilled water and bring to a boil.
2. Cool below 45°C.
3. Add 20 mL of iodine solution and mix well.

Following boiling, the prepared base can be stored for several weeks at 5 ±3°C, but once the iodine has been added, the media should be used the same day and any excess should be disposed of.

Specific recommendations for milk powder, chocolate, and cocoa-based samples

All milk powder, chocolate, or cocoa-based samples can benefit from an alternative enrichment.

Use prewarmed (37°C) sterile UHT skim milk supplemented with Brilliant Green (0.002%), instead of Buffered Peptone Water, as the enrichment media.

- Milk powder samples should be incubated at 37°C for 22 ±2 hours.
- Chocolate and cocoa-based samples should be incubated, as described in the protocol, at 37°C for 19 ±1 hour.

Specific recommendations for potentially acidic/alkaline samples

Samples which potentially deviate from neutral pH should be prepared as follows:

1. Dilute the sample according to the sample enrichment protocol.
2. Incubate for 60 ±5 minutes at room temperature.
3. Mix by hand massaging, and determine the pH.
4. If necessary, adjust the pH to 6.8 ±0.2, and mix well before determining the final pH.

B

Background

Product overview

Description of target microorganism

More than 2,400 *Salmonella* serotypes have been reported, all of which are potentially pathogenic. *Salmonella* is a frequently reported cause of foodborne illness, occurring in both epidemics and in isolated cases. *Salmonella* bacteria are the causative agent for Salmonellosis. Outbreaks have been associated with raw meats and poultry, eggs, milk and dairy products, seafood, coconut sauces, salad dressings, cocoa, chocolate, spices, frozen products, and vegetables such as hot peppers.

Audience

The Pathatrix® *Salmonella* spp. Kit is for professional use only and is intended for use by qualified users interested in determining the presence/absence of *Salmonella* spp. 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 removed for analysis 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.

Kit sensitivity

The sample preparation procedure allows you to detect as few as 1–10 cfu from 25–325 grams of food sample. The limitation of the Pathatrix® *Salmonella* spp. Kit is in the ability of the target to reproduce in the enrichment medium, be captured by the magnet, and subsequently be isolated on selective agar plates.

CAUTION! The Pathatrix® kit has been evaluated on cooked ham, chocolate, and chopped tomato food matrices. 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 to 40°C
Humidity	Maximum relative humidity 80% for temperatures up to 31°C, decreasing to 50%

AOAC Performance Tested Methods℠ Certification

The Pathatrix® *Salmonella* spp. Kit has been validated and certified by AOAC International Research Institute (License number 090203C) for the detection of *Salmonella* serovars from individual samples in cooked ham, chocolate, and chopped tomatoes. The test operates in conjunction with a standard Pathatrix® workflow. A standard 25-g food sample is hand mixed briefly with 225 mL of enrichment medium and incubated for a minimum of 16 hours at 37°C. After incubation, 10–50 mL of sample is subjected to a 15-minute Pathatrix® Auto Instrument capture cycle.

The method was validated for use with the following matrices:

- 25–325 g of cooked ham
- 25 g of chocolate
- 25 g of chopped tomatoes

C

Ordering Information

Related materials from Life Technologies

Equipment, consumables, and reagents	
Item	**Cat. no.**
Related consumable kits with associated beads	
Pathatrix® *Salmonella* spp. Kit — Same Day	APS50SD
Pathatrix® 5-Pooling *Salmonella* spp. Kit	APS250P
Pathatrix® 10-Pooling *Salmonella* spp. Kit	APS500P
Pathatrix® 5-Pool DUAL (*E. coli/Salmonella* spp.) Kit	APDES250P
Pathatrix® DUAL (*Listeria/Salmonella* spp.) Kit — Overnight	APD50
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
Consumables	
Foam filters	PFF
5-Pool Kit—Straws (254 mm) and Syringes (10 mL)	POOL510MLN
Reagents	
PBS, 10X, pH 7.4	AM9624 or AM9625
Related PCR assay	
MicroSEQ® *Salmonella* spp. Detection Kit	4403930

D

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. Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products. MLG 4.04. Microbiology Laboratory Guidebook.

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.

US FDA Bacteriological Analytical Manual (BAM), Chapter 5; go to **www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm** and scroll to *Salmonella*.

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