

# TaqMan® *Salmonella* Enteritidis Detection Kit

Egg and Environmental Testing Protocol

**Qualitative real-time PCR assay for the detection of Salmonella Enteritidis from pre-enriched poultry eggs and environmental samples. For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.**

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# About this Guide

## Safety information

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**Note:** For general safety information, see this Preface and [Appendix D, “Safety” on page 45](#). When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix for the complete alert on the chemical or instrument.

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### Safety alert words

Four safety alert words appear in Life Technologies Corporation user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined below:

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**IMPORTANT!** – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

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 **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

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 **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

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 **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

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Except for IMPORTANTs, each safety alert word in an Life Technologies Corporation document appears with an open triangle figure that contains a hazard symbol (see [“Safety information” on page 5](#)).

**SDSs** The SDSs for any chemicals supplied by Life Technologies Corporation or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see [“Obtaining SDSs” on page 48](#).

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**IMPORTANT!** For the SDSs of chemicals not distributed by Life Technologies Corporation or Ambion contact the chemical manufacturer.

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## How to use this guide

**User attention words** Two user attention words appear in Life Technologies Corporation user documentation. Each word implies a particular level of observation or action as described below:

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**Note:** – Provides information that may be of interest or help but is not critical to the use of the product.

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**IMPORTANT!** – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

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## How to obtain more information

See the following related documents for more information on the topics in this guide:

- *MagMAX™ Express-96 Magnetic Particle Processor User Manual* (Part no. N07849)
- *Plus/Minus Assay: Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System: Getting Started Guide* (Part no. 4347821)
- *PrepSEQ® Nucleic Acid Extraction Kit Protocol* (Part no. 4400739)

## How to obtain support

For the latest services and support information for all locations, go to:

[www.appliedbiosystems.com](http://www.appliedbiosystems.com)

At the Life Technologies Corporation web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems/Life Technologies Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Life Technologies Corporation user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.



# TaqMan<sup>®</sup> *Salmonella* Enteritidis Detection Kit

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# 1

This chapter covers:

- Product overview . . . . . 9
- Chemistry overview . . . . . 11
- Materials and equipment . . . . . 12

## Product overview

**Introduction** *Salmonella enterica* subsp. *enterica* serovar Enteritidis, or Salmonella Enteritidis (SE) is an important bacterial pathogen that has been linked to the consumption of eggs. A common organism in the digestive tract of poultry, SE can infect the reproductive tract of chickens and enter shell eggs before the shell is formed. Since the levels of SE in eggs from infected hens are often low, highly sensitive detection methods are required for fast and reliable detection of the pathogen.

The TaqMan<sup>®</sup> *Salmonella* Enteritidis Detection Kit offers the advantages of simplicity and quick results compared to traditional culture methods, in addition to being highly sensitive and specific. The PrepSEQ<sup>®</sup> Nucleic Acid Extraction sample preparation method described in this protocol is designed to work with most sample types, including whole-shell eggs and environmental samples. The PrepSEQ<sup>®</sup> Nucleic Acid Extraction method is automated on the MagMAX<sup>™</sup> Express-96 instrument for routine high-throughput testing.

**Intended use** The TaqMan® *Salmonella* Enteritidis Detection Kit is a real-time PCR assay to detect the presence of *Salmonella* Enteritidis DNA isolated from pre-enriched poultry egg and environmental samples.

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**IMPORTANT!** For Research Use Only. This product is not intended for any animal or human therapeutic or diagnostic use.

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**Product description** TaqMan® Pathogen Detection Kits provide a simple, reliable, and rapid procedure for detecting the presence of a specific bacterial pathogen. The assay uses the polymerase chain reaction (PCR) to amplify a target unique to that microorganism and TaqMan® probes that detect the presence of the specific organism.

**Sensitivity** The TaqMan® *Salmonella* Enteritidis Detection Kit assay can detect as little as 1 to 5 cfu of *Salmonella* Enteritidis from pre-enriched samples using the protocols provided in this document.

**Foods tested** The TaqMan® *Salmonella* Enteritidis Detection Kit has been validated for use in *Salmonella* Enteritidis detection from whole shell eggs, as well as from environmental samples.

**User assumptions** This document is intended for investigators testing for the presence of *Salmonella* Enteritidis in poultry egg and environmental samples.

**About this protocol** This protocol provides:

- Background material about the TaqMan® *Salmonella* Enteritidis Detection Kit
- A list of materials and equipment that can be used with the TaqMan® *Salmonella* Enteritidis Detection Kit
- Guidelines for sample enrichment and automated sample preparation using the MagMAX™ Express-96 instrument and the PrepSEQ® Nucleic Acid Extraction Kit
- Instructions for preparing PCR using the TaqMan® *Salmonella* Enteritidis Detection Kit on an Applied Biosystems 7500 Fast Sequence Detection System (SDS)
- General troubleshooting guidelines

**Operational conditions****Altitude**

The Applied Biosystems 7500 Fast Real-Time PCR System is for indoor use only and for altitudes not exceeding 2,000 m (6500 feet) above sea level.

**Temperature and humidity requirements**

Condition	Acceptable Range
Temperature	15 – 30°C (50 – 90°F) Maximum change of < 15°C (59°F) per 24 hours
Humidity	20 – 80% relative humidity, noncondensing

## Chemistry overview

**PCR Reaction components**

Reaction components include:

- *Salmonella* Enteritidis 10X Assay Mix
- 2X Environmental Master Mix 2.0
- Purified DNA from pre-enriched egg or environmental sample (user-supplied)

**Polymerase Chain Reaction (PCR)**

PCR is a method used to amplify, or increase the amount of, a specific DNA target sequence. Typically, the target DNA sequence is amplified using a reaction containing DNA polymerase, nucleotides, buffers, and primers complementary to that DNA sequence. When this solution is heated the DNA sequence denatures, separating it into separate strands. As the solution cools, the primers anneal, or bind, to the target sequences in the separated DNA strand. The DNA polymerase then creates a new strand by extending the primers with nucleotides, creating a copy of the DNA sequence. When repeated, this cycle of denaturing, annealing, and extending exponentially increases the number of target DNA sequences. Ideally, no amplification occurs if the target DNA sequence is not present.

**Fluorescence detection**

The TaqMan® probe contains a fluorescent dye on one end and a quencher (that suppresses fluorescence) on the other. If the target sequence is present during the PCR and the probe binds to the target DNA, then the probe is degraded, resulting in an increase in fluorescence. The instrument detects accumulation of PCR products by monitoring the increase in fluorescence. The increase in fluorescence signal is detected only if the target sequence is complementary to the probe and is amplified during PCR. Because of these requirements, separate nonspecific amplification is not detected.

**Internal Positive Control (IPC)**

Applied Biosystems includes an IPC in the TaqMan® Pathogen Detection Kits. An IPC in each reaction helps to avoid false negatives due to the presence of substances that inhibit PCR. The IPC also demonstrates whether or not PCR reagents are working and amplifying properly. This IPC eliminates the need for a positive control, reducing the risk of cross-contamination in unknown samples.

## Materials and equipment

**Kit contents**

The TaqMan® *Salmonella* Enteritidis Detection Kit (Part no. 4457030) contains reagents for 100 reactions. Components are shown in the table below.

Cap Color	Component Name	Volume (µL)
	10X <i>Salmonella</i> Enteritidis Target Assay Mix, 1 tube	300
	Negative Control, 1 tube	1,000
	2X Environmental Master Mix 2.0, 2 tubes	750

**Storage**

Follow the guidelines below for storing the TaqMan® *Salmonella* Enteritidis Detection Kit:

- On receipt, store the 10X Salmonella Enteritidis Target Assay Mix at  $-20^{\circ}\text{C}$  and protect from light. Excessive exposure to light may affect the fluorescent probes.
- Store the Negative Control and 2X Environmental Master Mix 2.0 at  $-2^{\circ}\text{C}$  to  $8^{\circ}\text{C}$ .
- Minimize freeze-thaw cycles.

**Shelf life** The kit expires one year after the date of manufacture.

**Materials and equipment not included** The following tables include equipment and materials for using the TaqMan<sup>®</sup> Salmonella Enteritidis Detection Kit. Unless otherwise indicated, many of the items listed are available from major laboratory suppliers (MLS).

#### Instruments from Applied Biosystems

Instruments	Source
Applied Biosystems 7500 Fast Real-Time PCR System	Contact your local Applied Biosystems sales office.
MagMAX <sup>™</sup> Express-96 Deep Well Magnetic Particle Processor	Contact your local Applied Biosystems sales office.

#### User-supplied materials

Materials	Source
MagMAX <sup>™</sup> Express-96 <sup>™</sup> Deep Well Tip Combs	Applied Biosystems (PN 4388487)
MagMAX <sup>™</sup> Express-96 Deep Well Plates	Applied Biosystems (PN 4388476)
MagMAX <sup>™</sup> Express-96 Standard Plates	Applied Biosystems (PN 4388475)

<b>Materials</b> <i>(continued)</i>	<b>Source</b>
MicroAmp® Fast Optical 96-well Reaction Plate (0.1 mL)	Applied Biosystems (PN 4346906)
MicroAmp® Optical Adhesive Film, PCR compatible	Applied Biosystems (PN 4311971)
MicroAmp® Clear Adhesive Film	Applied Biosystems (PN 4306311)
PrepSEQ® Nucleic Acid Extraction Kit	Applied Biosystems (PN 4428176)
(Optional) 96-Well Magnetic-Ring Stand	Applied Biosystems (PN AM10050)
Nuclease-Free Water	Applied Biosystems (PN AM9938)
Incubators, 37 ± 2°C	MLS
Microcentrifuge (Eppendorf 5415D or equivalent)	MLS
Plate Centrifuge (Eppendorf 5804 or equivalent)	MLS
Dry Heat Block 37°C	MLS
Microcentrifuge Tubes, PCR clean, 1.5 ml	(MLS)
Disposable Gloves	(MLS)
Pipettors i.e. Positive-displacement/air displacement/multichannel	(MLS)
Micropipette tips, aerosol-resistant	(MLS)
Sample Bags (Whirl-Pak® bags or equivalent)	(MLS)
Trypticase Soy Broth	(MLS)
Ethanol, 95%	(MLS)
Isopropanol	(MLS)
Vortexer	(MLS)

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<b>Materials</b> <i>(continued)</i>	<b>Source</b>
Tetrathionate Broth	(MLS)
4 inch × 4 inch 12-ply sterile gauze	(MLS)
Canned evaporated, canned skim evaporated, or canned low-fat evaporated milk	(MLS)



# Protocol for Detecting *Salmonella* Enteritidis in Egg and Environmental Samples

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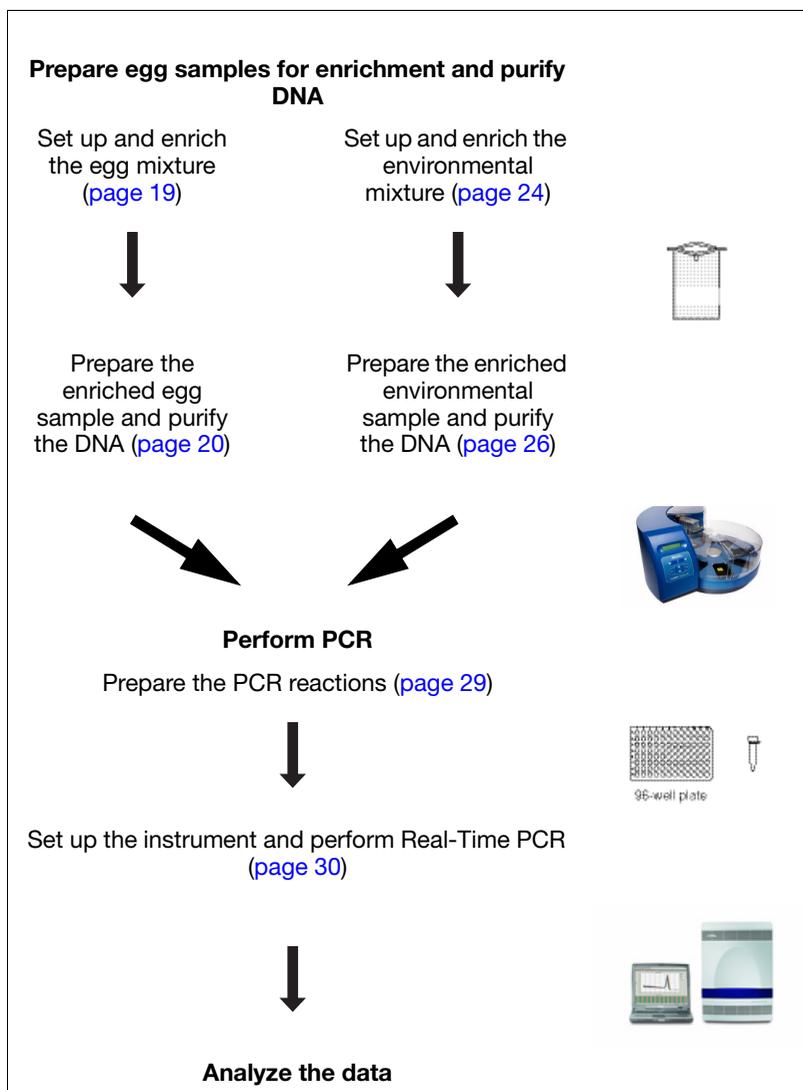
# 2

This chapter covers:

- Workflow . . . . . 18
- Prepare egg samples for enrichment and purify DNA . . . . . 19
- Prepare pre-enriched environmental samples and purify DNA. . . . . 24
- Perform PCR . . . . . 29
- Analyze the data . . . . . 36

## Workflow

The following workflow describes sample preparation for an egg mixture or for an environmental mixture, then steps to perform PCR using either sample type when using the TaqMan<sup>®</sup> *Salmonella* Enteritidis Detection Kit.



# Prepare egg samples for enrichment and purify DNA

**Overview** **IMPORTANT!** Perform these steps for **egg** samples only. To enrich and prepare environmental samples, go to “[Prepare pre-enriched environmental samples and purify DNA](#)” on page 24.

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Sample setup and enrichment for the target organism is the first step in using the TaqMan<sup>®</sup> *Salmonella* Enteritidis Detection Kit to detect *Salmonella* Enteritidis in egg samples. Sample preparation, which is the second step, consists of preparing the enriched sample for inclusion in PCR.

The PrepSEQ<sup>®</sup> Nucleic Acid Extraction Kit (Part no. 4428176) is used for DNA extraction and sample preparation. Refer to the *PrepSEQ<sup>®</sup> Nucleic Acid Extraction Kit Protocol* (Part no. 4400739) for information about kit contents and storage.

For all chemicals, read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Set up and enrich the egg mixture

**IMPORTANT!** The instructions below are specifically for detection of *Salmonella* Enteritidis in egg samples. Do **not** follow the instructions in the *PrepSEQ<sup>®</sup> Nucleic Acid Extraction Kit Protocol*.

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### Set up the egg mixture

Whole shell eggs must be disinfected and pooled as described in the FDA BAM [Andrews, W.H. and Hammack, T.S. 2007. Chapter 5, *Salmonella*. In *Bacteriological Analytical Manual (BAM)*. U.S. Food and Drug Administration, available at [www.fda.gov](http://www.fda.gov)].

1. Disinfect eggs:
  - a. Prepare a solution of 3 parts 70% ethanol to 1 part iodine/potassium iodide solution with a 3:1 solution consisting of 3 parts of 70% ethanol to 1 part iodine/potassium iodide solution.

- b. Soak the eggs for 10 seconds and allow them to air-dry at room temperature (approximately 20 to 25°C).
2. Aseptically crack 20 eggs (approximately 1 L) into each sample bag.
3. Mix the contents until the eggs yolks are broken.

#### Enrich the egg mixture

1. Add 0.1 volume ( $\pm 20\%$ ) of 10 $\times$  Trypticase Soy Broth (TSB) to each egg pool.

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**Note:** A pool of 20 eggs contains approximately 1 L of egg. Add approximately 100 mL of 10 $\times$  TSB to each egg pool.

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2. Mix the solution by shaking until the broth is homogeneously dispersed in the egg pool.
3. Incubate the mixture at  $37 \pm 2^\circ\text{C}$  for 24 hours.

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**Note:** After the incubation is complete, proceed immediately to [“Prepare the enriched egg sample and purify the DNA”](#).

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#### Prepare the enriched egg sample and purify the DNA

#### Set up the reagent plates

The reaction requires these components, which are included in the PrepSEQ<sup>®</sup> Nucleic Acid Extraction Kit:

- Lysis Buffer
- Binding Solution
- Elution Buffer
- Wash Buffer concentrate
- Magnetic particles

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**IMPORTANT!** Do not use PK for egg samples, although the PrepSEQ<sup>®</sup> Kit also contains PK and PK buffer.

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1. Clean the lab bench, pipettors, and MagMAX™ Express-96 processor with 10% bleach solution and wipe until dry.
2. Before using a new PrepSEQ® kit, prepare reagents and beads:
  - **Wash Buffer:** Add 74 mL of 95% ethanol into the Wash Buffer Concentrate container.
  - **Binding Solution:** Add 35 mL of 100% isopropanol into the Binding Solution container.
  - **Magnetic Particles:** Incubate the Magnetic Particles at 37°C for at least 10 minutes. Make sure to incubate the particles until no white precipitate is present, and to vortex the particles vigorously before each use, to ensure that all salts are dissolved.

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**Note:** If after 10 minutes the white precipitate is not completely dissolved, a longer incubation time and higher temperatures (up to 50°C) can be used. After incubating, vortex to completely resuspend the particles.

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3. Prepare the reagent plates as follows:
  - a. **Tip Comb:** Place a tip comb in a MagMAX™ Express-96 Express-96 Standard plate.
  - b. **Lysis Plate:** In a MagMAX™ Express-96 Deep Well plate, add 250 µL of Lysis Buffer into one well for each sample to analyze, plus one well for buffer control (BC).
  - c. **Elution Plate:** In a MagMAX™ Express-96 Standard plate, add 100 µL of Elution Buffer into each well that corresponds to a sample well in the Lysis plate.
  - d. **Wash Plates:** Prepare 2 identical Wash Plates in MagMAX™ Express-96 Deep Well plates by adding 300 µL of Wash Buffer to well positions that contain elution buffer in the elution plate.

### Set up the lysis plate

1. Collect the enriched sample from the incubator and briefly shake the Whirl-Pak® bag to mix the sample.

2. In each well of the Lysis Plate that contains Lysis Buffer except the BC well, add 250  $\mu$ L of sample.
3. Examine the Magnetic Particles to make sure no white precipitate is present after incubation. If white precipitate is present, continue to incubate the magnetic particles until white precipitate is completely dissolved.
4. Vortex the Magnetic Particles for 5 seconds until resuspension is complete.
5. In each well of the plate that contains buffer (including the BC well), add 30  $\mu$ L of Magnetic Particles.
6. In each well of the plate that contains buffer (including the BC well), add 300  $\mu$ L of Binding Solution.

#### Load and process the plates

1. Power on the MagMAX<sup>™</sup> Express-96 Magnetic Particle Processor.
2. Select program **4428176DWPrepSEQFA**, using the Up-arrow and Down-arrow keys. Press **Start**.
3. Load the plates into the MagMAX<sup>™</sup> Express-96 processor according to the instrument readout. Verify that their orientation is {A1 to A1}.
  - a. **Tip comb:** Load the tip comb, then press **Start**.
  - b. **Elution plate:** Load the plate, then press **Start**.
  - c. **Wash plate 2:** Load the plate, then press **Start**.
  - d. **Wash plate 1:** Load the plate, then press **Start**.
  - e. **Lysis plate:** Load the plate, then press **Start**.

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**Note:** See “[Set up the reagent plates](#)” on page 20 for a description of reagent plate preparation. See “[Set up the lysis plate](#)” on page 21 for a description of lysis plate preparation.

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4. When the sample preparation is complete, the message “Enjoy your DNA” displays on the screen. Remove the Elution Plate from the instrument.

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STOPPING POINT. Proceed immediately to [“Perform PCR” on page 29](#) or store the elution plate at  $-20^{\circ}\text{C}$  until PCR.

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## Prepare pre-enriched environmental samples and purify DNA

**Overview** **IMPORTANT!** Perform these steps for **environmental** samples only. To enrich and prepare egg samples, go to “[Prepare egg samples for enrichment and purify DNA](#)” on page 19.

---

Sample setup and enrichment for the target organism is the first step in using the TaqMan<sup>®</sup> *Salmonella* Enteritidis Detection Kit to detect *Salmonella* Enteritidis in environmental samples. Sample preparation, which is the second step, consists of preparing the pre-enriched sample for inclusion in PCR.

The PrepSEQ<sup>®</sup> Nucleic Acid Extraction Kit (Part no. 4428176) is used for DNA extraction and sample preparation. Refer to the *PrepSEQ<sup>®</sup> Nucleic Acid Extraction Kit Protocol* (Part no. 4400739) for information about kit contents and storage.

For all chemicals, read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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### Set up and enrich the environmental sample

**IMPORTANT!** The instructions below are specifically for detection of *Salmonella* Enteritidis in environmental samples. Do **not** follow the instructions in the *PrepSEQ<sup>®</sup> Nucleic Acid Extraction Kit Protocol*.

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#### Set up the environmental mixture

Environmental sampling and preparation must follow the guidelines outlined in the U.S. FDA protocol *Environmental Sampling and Detection of Salmonella in Poultry Houses*, October 2008 Edition 3, available at [www.fda.gov](http://www.fda.gov). Manure is the preferred sample type.

Perform the following steps, using a separate gauze pad and Whirl-Pak<sup>®</sup> bag for each row/bank:

1. Aseptically affix a 10 cm × 10 cm (4 in × 4 in) 12-ply sterile gauze pad to a manure drag pole, using sterile clips or string.

2. Moisten the pad with canned evaporated milk, canned skim (fat free) evaporated milk, or canned low-fat evaporated milk.
3. Drag the moistened gauze pad over the manure the entire length of one side of row or bank.
4. Remove the gauze pad from the pole.
5. Affix a clean pad to the pole.
6. Drag the other side of the row/bank with a second gauze pad over the other side of the row or bank.
7. Repeat [steps 1](#) through [6](#) for all rows or banks of the house.
8. Place each gauze pad into a separate Whirl-Pak<sup>®</sup> bag. Add sufficient milk to keep the pad wet (no more than a tablespoon or approximately 15 mL).

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**IMPORTANT!** Be sure to put each pad into a separate bag.

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### Enrich the environmental mixture

For each sample bag:

1. Draw into a pipette a volume of tetrathionate enrichment broth that is 9 times the volume of the sample in the sample bag. Add the broth to the sample to produce a 1:9 sample:enrichment broth solution.
2. Mix the solution by shaking the bag vigorously up and down at least 10 times in a 30 cm (1 ft) arc in approximately 30 seconds.
3. Incubate the bag at 37°C ± 2°C for 24 hours.

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**Note:** After the incubation is complete, proceed immediately to [“Prepare the enriched environmental sample and purify the DNA”](#).

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**Prepare the enriched environmental sample and purify the DNA**

**Set up the reaction components**

The reaction requires these components, which are included in the PrepSEQ<sup>®</sup> Nucleic Acid Extraction Kit:

- Lysis Buffer
- Binding Solution
- Elution Buffer
- Wash Buffer concentrate
- Magnetic particles
- Proteinase K (PK) buffer
- Proteinase K (PK)

1. Clean the lab bench, pipettors, and MagMAX<sup>™</sup> Express-96 processor with 10% bleach solution and wipe until dry.
2. Before using a new PrepSEQ<sup>®</sup> kit, prepare the reagents and beads:
  - **Wash Buffer:** Add 74 mL of 95% ethanol into the Wash Buffer Concentrate container.
  - **Binding Solution:** Add 35 mL of 100% isopropanol into the Binding Solution container.
  - **Magnetic Particles:** Incubate the Magnetic Particles at 37°C for 10 minutes. Make sure to vortex the particles vigorously before each use, to ensure that all salts are dissolved.

**Incubate the samples and PK**

For each sample:

1. Collect the enriched sample from the incubator and briefly shake the Whirl-Pak<sup>®</sup> bag to mix the sample.
2. Create the Lysis Plate:
  - a. Create the PK mix:

In a MagMAX<sup>™</sup> Express-96 Deep Well plate, in one well for each sample to analyze, plus one buffer control (BC) well, add:

- 140  $\mu$ L of Proteinase K Buffer and
  - 10  $\mu$ L of Proteinase K
- b. In each well that contains PK mix (except the BC well), add 250  $\mu$ L of sample. Mix by pipetting up and down 5 times.
3. Incubate the Lysis Plate at room temperature (approximately 20 to 25 °C) for 10 to 15 minutes.
- During the incubation, set up the MagMAX™ Express-96 particle processor for sample preparation.

### Set up and load the reagent plates

1. Prepare the reagent plates as follows:
  - a. **Tip Comb:** Place a tip comb in a MagMAX™ Express-96 Standard plate.
  - b. **Elution Plate:** Add 100  $\mu$ L of Elution Buffer to each well of a MagMAX™ Express-96 Standard plate that corresponds to those wells that contain sample in the Lysis Plate.
  - c. **Wash Plates:** Prepare 2 identical Wash Plates in MagMAX™ Express-96 Deep Well plates by adding 300  $\mu$ L of Wash Buffer to well positions that contain elution buffer in the elution plate.
2. Power on the MagMAX™ Express-96 Magnetic Particle Processor.
3. Select program **4428176DWPRepSEQFA**, using the Up-arrow and Down-arrow keys. Press **Start**.
4. Load the Elution Plate and Wash Plates into the MagMAX™ Express-96 processor according to the instrument readout. Verify that their orientation is {A1 to A1}.
  - a. **Tip comb:** Load the tip comb, then press **Start**.
  - b. **Elution plate:** Load the plate, then press **Start**.
  - c. **Wash plate 2:** Load the plate, then press **Start**.

d. **Wash plate 1:** Load the plate, then press **Start**.

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**Note:** See [step 1 on page 27](#) for a description of reagent plate preparation.

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#### Set up and load the lysis plate

1. Vortex the Magnetic Particles for 5 seconds until resuspension is complete.
2. When incubation of the PK and sample in the Lysis plate is complete (see [page 27](#)), add the components shown below into each well in the Lysis plate that contains sample, and into the BC well. Pipette up and down 5 times to mix.

Component	Volume
Lysis buffer	250 $\mu$ L
Magnetic Particles	30 $\mu$ L
Binding Solution	325 $\mu$ L

3. Load the Lysis Plate into the instrument. Press **Start**.
4. When the sample preparation is complete, the message “Enjoy your DNA” is displayed on the screen. Remove the Elution Plate from the instrument.

---

**STOPPING POINT.** Proceed immediately to [“Perform PCR” on page 29](#) or store the elution plate at  $-20^{\circ}\text{C}$  until PCR.

---

# Perform PCR

**Overview** Preparing the PCR run is the third step in using the TaqMan<sup>®</sup> *Salmonella* Enteritidis Detection Kit. Preparing the PCR run consists of creating the plate document and the reaction mix.

**Note:** TaqMan<sup>®</sup> *Salmonella* Enteritidis Detection Kits run with a standard PCR protocol.

For all chemicals, read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Prepare the PCR reactions

1. Thaw all reagents and samples. The table shows the reagents and volumes for each PCR reaction.

**Note:** It might be necessary to remove condensation from DNA samples after thawing and before opening the samples, to avoid cross contamination. Centrifuge DNA samples (in 96-well plates) in a plate centrifuge at 2,000 x g for 1 minute.

2. Prepare PCR reagent mix:
  - a. For each sample well, one BC well, and one negative control well, combine the appropriate volumes of the reagents shown in the table below.

Component	Volume per well <sup>‡</sup>
2XEnvironmental Master Mix 2.0	16.5 µL
<i>Salmonella</i> Enteritidis 10X Assay Mix	3.3 µL

<sup>‡</sup> Volumes include 10% overage.

- b. Vortex the reagent mix for approximately 5 seconds to mix thoroughly. Use immediately or keep on ice until ready to use.

- In a 96-well Fast PCR plate, add the components to the wells as shown in the following table. Mix by pipetting up and down.

Well(s)	Component 1		Component 2	
	Reagent	Volume per well	Reagent	Volume per well
Sample wells	PCR reagent mix	18 $\mu$ L	Sample	12 $\mu$ L
Negative control	PCR reagent mix	18 $\mu$ L	Pathogen Negative Control	12 $\mu$ L
Buffer control	PCR reagent mix	18 $\mu$ L	Buffer Control	12 $\mu$ L

- Cover the plate with optical adhesive film. Use the film applicator to form a tight seal.
- Vortex the plate for 5 seconds, then centrifuge it in a plate centrifuge at 2,000 x g for 1 minute.

**Set up the instrument and perform Real-Time PCR**

- Turn on the 7500 Fast instrument by pressing the power button in the lower right corner. Turn on the laptop computer by pressing the power button.
- Open the SDS (Sequence Detection Software) software by clicking the SDS icon.

3. In the SDS Software, select **File ▶ New** to create a new document.

**New Document Wizard**

**Define Document**  
Select the assay, container, and template for the document, and enter the operator name and comments.

Assay: Standard Curve (Absolute Quantitation)

Container: 96-Well Clear

Template: Blank Document

Run Mode: Standard 7500

Operator: Administrator

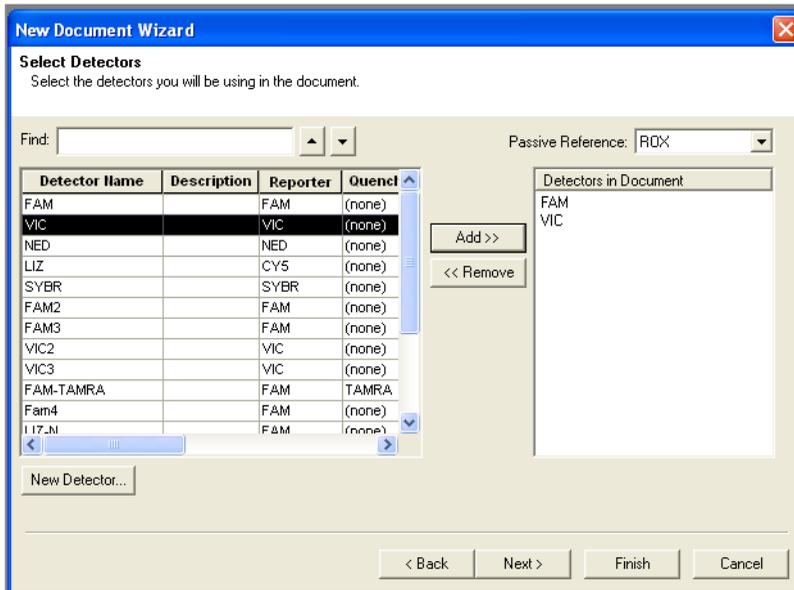
Comments: SDS v1.4

Plate Name: Plate1

< Back   Next >   Finish   Cancel

4. In the Define Document screen, set up the run file as follows, then click **Next**.
  - Assay: **Standard Curve** (Absolute Quantitation)
  - Container: **96-Well Clear**
  - Run Mode: **Standard 7500**
  - Operator, Comments, and Plate Name: Add information as desired

5. In the Select Detectors page, select (or create) detectors for FAM™ and VIC® dyes (the Quencher Dye should be set to “None” or “Non Fluorescent”). Add the detectors to the document. Click **Next**.



- Select the wells that contain sample and add FAM<sup>™</sup> and VIC<sup>®</sup> detectors as shown below. Click **Next**.

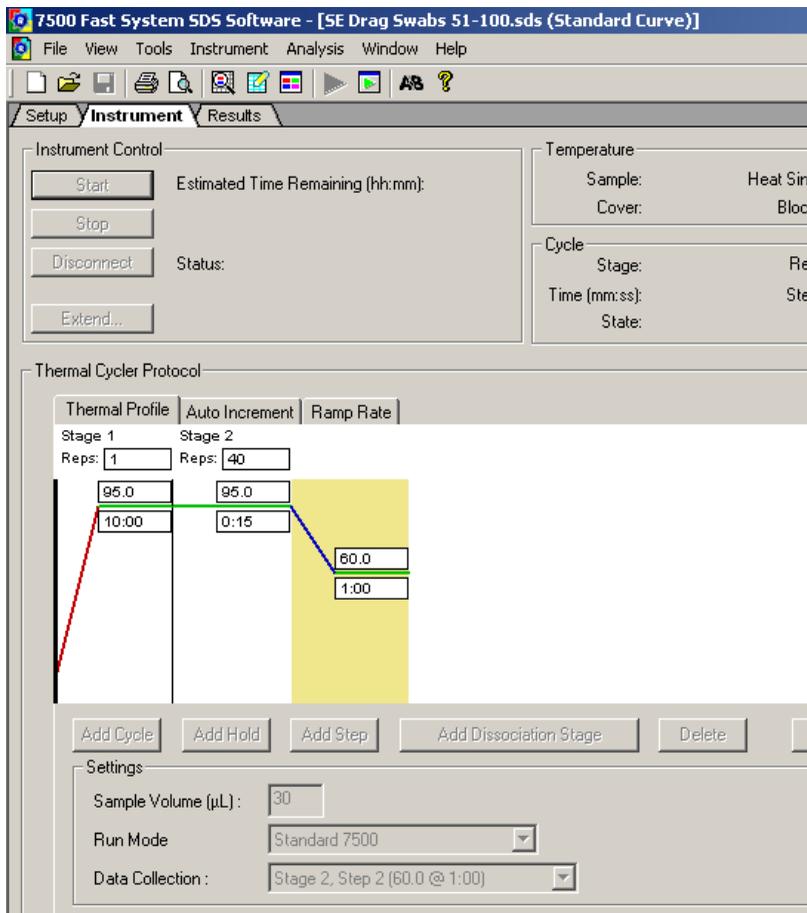
	1	2	3	4	5	6	7	8	9	10
A	Drag Swab-51 U	Drag Swab-52 U	Drag Swab-53 U	Drag Swab-54 U	Drag Swab-55 U	Drag Swab-56 U	Drag Swab-57 U	Drag Swab-58 U	Drag Swab-59 U	Drag Swab-60 U
B	Drag Swab-61 U	Drag Swab-62 U	Drag Swab-63 U	Drag Swab-64 U	Drag Swab-65 U	Drag Swab-66 U	Drag Swab-67 U	Drag Swab-68 U	Drag Swab-69 U	Drag Swab-70 U
C	Drag Swab-71 U	Drag Swab-72 U	Drag Swab-73 U	Drag Swab-74 U	Drag Swab-75 U	Drag Swab-76 U	Drag Swab-77 U	Drag Swab-78 U	Drag Swab-79 U	Drag Swab-80 U
D	Drag Swab-81 U	Drag Swab-82 U	Drag Swab-83 U	Drag Swab-84 U	Drag Swab-85 U	Drag Swab-86 U	Drag Swab-87 U	Drag Swab-88 U	Drag Swab-89 U	Drag Swab-90 U
E	Drag Swab-91 U	Drag Swab-92 U	Drag Swab-93 U	Drag Swab-94 U	Drag Swab-95 U	Drag Swab-96 U	Drag Swab-97 U	Drag Swab-98 U	Drag Swab-99 U	Drag Swab-100 U
F										
G										

**IMPORTANT!** Each well that contains sample must display a colored “U” symbol after this step to indicate the FAM<sup>™</sup> and VIC<sup>®</sup> dyes are associated with the wells.

**Note:** The FAM<sup>™</sup> dye is used to detect *Salmonella* Enteritidis, and the VIC<sup>®</sup> dye is used to detect the internal positive control.

- Enter the sample names as they appear in the 96-well format. Click **Next**.

8. Select the **Instrument** tab. Verify that the Run mode is set to “Standard 7500”. Verify that the thermal cycling conditions are as shown:
  - Thermal Cycler Protocol Window:
    - Stage 1: 1 cycle (95°C for 10:00)
    - Stage 2: 40 cycles (95°C for 0:15, 60°C for 1:00)
  - Settings Window:
    - Sample Volume (µL): 30
    - Run Mode: Standard 7500
    - Data Collection: Stage 2, Step 2 (60.0 @ 1:00)



9. Save the SDS document (we recommend DDMMYY format).
10. Place the 96-well Fast PCR plate into the instrument and click **Start**. The instrument displays the run status during the run including the estimated run time.

## Analyze the data

View the amplification plots for all reactions and check each sample for FAM™ and VIC® dye signals. We recommend using the default baseline and threshold values in the SDS analysis software. When the run is complete and the amplification plots are displayed, click **Analyze**.

Guidelines for interpreting results		
FAM™ dye	VIC® dye	Interpretation
+	+	Positive for SE
+	-	Positive for SE
-	+	Negative for SE
-	-	Inconclusive

We do not recommend using the same method to screen samples and to confirm the results. The TaqMan® Pathogen Detection System is used to screen samples. We recommend biochemical methods to confirm positive results. The FDA BAM [*Bacteriological Analytical Manual (BAM)*], available at [www.fda.gov](http://www.fda.gov)] manual for egg and environmental testing contains approved protocols for culture confirmation.

For more information about analyzing your data, refer to your real-time PCR instrument user guide.

# Troubleshooting



Observation	Possible Cause	Action
<p>No IPC signal and no target-specific signal is detected in sample wells.</p> <p><b>Note:</b> When there is a target-specific signal in the absence of IPC signal for an unknown sample, the result is considered positive and no troubleshooting is required.</p>	<p>PCR inhibition due to presence of magnetic particles in the elution plate during sample preparation.</p>	<p>Avoid disturbing magnetic particles during transfer of eluted DNA sample to the assay reaction.</p> <p>Optional:</p> <ul style="list-style-type: none"> <li>• Spin the elution plate for 30 seconds at 4,000 x <i>g</i> to pellet the magnetic particles to the bottom of the plate.</li> <li><i>or</i></li> <li>• Place the elution plate on the 96-well magnetic ring stand during transfer of the sample to the assay.</li> </ul>
	<p>Elution plate contains incompletely removed particulate residue from the food sample.</p>	<p>Avoid residue during transfer of eluted DNA to the assay.</p> <p>Optional: Spin the elution plate for 30 seconds at 4,000 x <i>g</i> to pellet food debris to the bottom of the wells.</p>
	<p>PCR inhibition due to incomplete removal of inhibitors.</p>	<p>If PCR is still inhibited, dilute the sample (for example, 1:5 or 1:10) to dilute inhibitors.</p> <p>Repeat sample preparation, then repeat assay.</p>
	<p>Environmental Master Mix not stored correctly.</p>	<p>Repeat the assay using properly stored assay components.</p>
	<p>10X Target Assay Mix not stored correctly.</p>	<p>Avoid freezing and thawing assay components. Protect the Assay Mix from light.</p>

<b>Observation</b> <i>(continued)</i>	<b>Possible Cause</b>	<b>Action</b>
<p>No IPC signal and no target-specific signal is detected in sample wells <i>(continued)</i>.</p> <p><b>Note:</b> When there is a target-specific signal in the absence of IPC signal for an unknown sample, the result is considered positive and no troubleshooting is required.</p>	<p>Pipetting Error (no premix solution added).</p> <p>Dye was not assigned to sample wells during instrument setup.</p>	<p>Repeat the assay. Make sure to pipet premix solution into all wells.</p> <p>Click on the Setup tab and add the FAM™ and VIC® dyes to each well that contains sample, then reanalyze the data by clicking the green arrow in the toolbar at the top of the page.</p>
<p>No IPC is detected, but target-specific signal is detected in sample wells.</p>	<p>High copy number of target DNA in samples causes preferential amplification of the target-specific DNA.</p>	<p>The test is positive.</p>
<p>Target-specific signal is detected in negative-control wells.</p>	<p>Carryover contamination.</p>	<p>Repeat the assay using fresh reagents and clean pipetting equipment.</p> <p>If negative control still shows contamination, repeat assay with a new kit.</p> <p>If negative control still shows contamination, contact Applied Biosystems/Life Technologies Technical Support.</p>
<p>In negative control wells, target-specific signal is detected, but no IPC is detected.</p>	<p>Carry-over contamination and one of the following:</p> <ul style="list-style-type: none"> <li>• High copy number of target DNA in samples causing preferential amplification of the target-specific DNA.</li> <li>or</li> <li>• Problem with IPC amplification.</li> </ul>	<p>Examine the sample wells to determine whether the IPC signal is present.</p> <p>If IPC signal is present in the sample wells, IPC amplification is not the cause. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.</p>
<p>No target-specific signal detected in positive control wells.</p>	<p>No positive control in positive control wells.</p>	<p>Repeat the assay. Make sure to pipet positive control into all positive control wells.</p>

<b>Observation</b> <i>(continued)</i>	<b>Possible Cause</b>	<b>Action</b>
Results for a sample and replicate(s) are both positive and negative.	Sample(s) or reagent(s) not stored correctly.	If you ran only two replicates and results are not consistent, repeat the assay using fresh samples and reagents.
	DNA concentration is very low.	Repeat DNA purification using the pre-enrichment media (i.e., do not perform enrichment). Repeat PCR using fresh reagents.



# Preventing Contamination

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# B

## Overview

PCR assays require special laboratory practices to avoid false positive amplifications (Kwok and Higuchi, 1989). The high sensitivity of these assays can lead to amplification of a single DNA molecule (Saiki *et al.*, 1985; Mullis and Faloona, 1987).

## Best practices for nucleic acid extraction and PCR

- If possible, maintain separate work areas, dedicated equipment, and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products

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**Note:** Rooms can be simulated using a clean bench or PCR bench available from major laboratory suppliers.

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- Do not bring amplified PCR products into the PCR setup area.
- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves at all times.
- Change gloves whenever you suspect that they are contaminated and before leaving the work area, to protect yourself from contaminating and pathogenic materials.
- To avoid false positives due to cross-contamination, do not include a positive control unless required. If a positive control is necessary, close all unknown sample tubes before pipetting the positive control.

- Open and close all sample tubes and reaction plates carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use positive-displacement pipettes or aerosol-resistant pipette tips.
- Do not re-use pipette tips or put once-used tips into reagents. During sample preparation, use a separate set of pipettes for handling pre-enrichment cultures and clean reagents.
- Before working with pathogenic bacteria, clean the lab bench and pipettors with 10% bleach and wipe to dryness.
- We also recommend cleaning the MagMAX™ Express-96 instrument with 10% bleach solution and wiping to dryness before and after each use.
- Clean lab benches and equipment after use with freshly diluted 10% bleach solution.

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**IMPORTANT!** To avoid false positives due to amplified material in your work area, do not open tubes after amplification.

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## Plate layout suggestions

- If you use a positive control, handle it extremely carefully!
- If possible, put positive controls in one of the outer rows or columns.
- If possible, separate negative and positive controls by one well.
- If possible, put at least one well between unknown samples and controls.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.

# References

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U.S. Food and Drug Administration, *Bacteriological Analytical Manual (BAM)*, available at [www.fda.gov](http://www.fda.gov)

U.S. Food and Drug Administration, Oct. 2008. *Environmental Sampling and Detection of Salmonella in Poultry Houses*, Edition 3, available at [www.fda.gov](http://www.fda.gov)

Kwok, S. and Higuchi, R. 1989. Avoiding false positives with PCR. *Nature* 339:237-238.

Mullis, K.B. and Faloona, F.A. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 155:335-350.

Saiki, R.K., Scharf, S., Faloona, F., et al. 1985. Enzymatic amplification of  $\beta$ -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350-1354.



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# Chemical safety

## General chemical safety

### Chemical hazard warning



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**WARNING! CHEMICAL HAZARD.** Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

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**WARNING! CHEMICAL HAZARD.** All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

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**WARNING! CHEMICAL HAZARD.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

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**WARNING! CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

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### Chemical safety guidelines

To minimize the hazards of chemicals:

- 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. (See [“About SDSs” on page 48.](#))

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.



## SDSs

**About SDSs** Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

**Obtaining SDSs** The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

1. Go to [www.appliedbiosystems.com](http://www.appliedbiosystems.com), click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
  - **Open** – To view the document
  - **Print Target** – To print the document
  - **Save Target As** – To download a PDF version of the document to a destination that you choose

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**Note:** For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

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## Chemical waste safety

### Chemical waste hazards



**CAUTION! HAZARDOUS WASTE.** Refer to Safety Data Sheets and local regulations for handling and disposal.



**WARNING! CHEMICAL WASTE HAZARD.** Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.



**WARNING! CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

### Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.



- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

## Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

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**IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

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## Biological hazard safety

### General biohazard



**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:

- U.S. Department of Health and Human Services guidelines found at:  
<http://www.cdc.gov/biosafety/publications/index.htm>
- 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](http://www.cdc.gov)

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## Safety alerts

### Chemical alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see [“Safety alert words” on page 5](#).

### General alerts for all chemicals

Read and understand the Safety Data Sheets (SDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. Wear appropriate protective eyewear, clothing, and gloves.

# Glossary

<b>Amplification</b>	The process of making copies of, and thereby increasing the amount of, a specific DNA sequence.
<b>Environmental Master Mix 2.0 (EMM 2.0)</b>	A common reagent for pathogen detection assays. The EMM 2.0 is used to prepare the premix solution. It contains the polymerase enzyme that initiates PCR in the presence of the necessary primers and DNA sample.
<b>Internal Positive Control (IPC)</b>	A control present in all reaction wells (contained in the Target Assay Mix). The IPC should always yield a positive result. If it does not, there may be a problem with amplification.
<b>Negative control (NC)</b>	A control that monitors for contamination (unexpected amplification in the absence of a target) and reagent integrity (IPC signal should be present). One negative control is required for each target assay type per run.
<b>Polymerase Chain Reaction (PCR)</b>	Technology used to amplify, or increase the amount of, a target DNA sequence.
<b>Positive control</b>	A control that monitors for the expected amplification of a target. Target signal not detected in a positive control well indicates a pipetting error or a problem with amplification. A positive control is optional for each target assay and should be used with precautions, because it can cause cross-contamination.
<b>Premix solution</b>	A solution that contains Environmental Master Mix 2.0 (EMM 2.0) and Target Assay Mix (TAM).
<b>Primer</b>	A segment of DNA that is complementary to the target DNA sequence or Internal Positive Control DNA sequence. Needed to initiate amplification.

<b>Probe</b>	A segment of DNA that is complementary to the target DNA sequence or Internal Positive Control DNA sequence. The probe is labeled with a reporter dye. When the probe binds to the target or Internal Positive Control, a reaction detected by the Sequence Detection System (SDS) or Real-Time PCR System indicates the presence of the target or Internal Positive Control.
<b>Target</b>	The pathogen being tested.
<b>Target Assay Mix (TAM)</b>	Target-specific reaction for the pathogen detection assay. TAM is used to prepare the premix solution. TAM contains specific primers and probes for the target and the IPC, as well as IPC DNA template.
<b>Unknown sample</b>	A DNA sample from a substance that you are testing for the presence of one or more pathogens.



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