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MicroSEQ® *Salmonella* spp. Detection Kit

Protocol

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 031001

BY AFNOR CERTIFICATION
NF
VALIDATION
EN ISO 16140

ABI 29/02 – 09/10

[End of validity: refer to certificate available at www.afnor-validation.com]

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

Certified by AFNOR Certification

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Part Number 4405964 Rev. C
10/2011

Contents

Safety information

Note: For general safety information, see this Preface and Appendix B, "Safety" on page 29. 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.

Safety alert words

Four safety alert words appear in Applied Biosystems 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:

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

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.

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

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.

SDSs

The SDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see Appendix B.

IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

How to use this guide

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

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

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

How to obtain support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**.

At the Applied Biosystems web site, you can:

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

MicroSEQ® *Salmonella* spp. Detection Kit

Product overview

Product description

The MicroSEQ® *Salmonella* spp. Detection Kit detects *Salmonella* spp. simply, reliably, and rapidly in food samples using a lyophilized reagent format. The assay uses the polymerase chain reaction (PCR) to amplify a unique microorganism-specific DNA target sequence and a TaqMan® probe to detect the amplified sequence.

Description of target microorganisms

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.

Kit sensitivity

The sensitivity of the assay in real culture samples depends on the quality of the sample preparation method that is used. The sample preparation procedures in the *PrepSEQ® Nucleic Acid Extraction Kit Protocol*: *Salmonella spp.* and *PrepSEQ® Rapid Spin Sample Preparation Kit Protocol*: *Salmonella spp.* allow you to detect 1 to 3 colony-forming units (CFU) in 25 grams of food for all the matrices in AOAC study. The limit of detection is 10^3 cfu/mL (not part of AOAC study).

Kit specificity

The MicroSEQ® *Salmonella* spp. Detection Kit can detect all *Salmonella enterica* species tested and did not detect any non-*Salmonella* species tested. The method does not allow detection of *Salmonella bongori*.

Audience

This document is intended for microbiological analysts who need to test for *Salmonella* spp. in food.

AOAC Performance Tested Methods℠ Certification

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 031001

The MicroSEQ® *Salmonella* spp. Detection kit earned the Performance Tested Methods℠ Certification from the AOAC Research Institute. The validation was conducted using ISO 6579 as the reference method. The validated workflow includes:

- Two sample preparation kit options:
  - The PrepSEQ® Nucleic Acid Extraction Kit
  - The PrepSEQ® Rapid Spin Sample Preparation Kit
- The MicroSEQ® *Salmonella* spp. Detection Kit
- The Applied Biosystems 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software v1.1

The workflow was certified for use with the following:

- Food matrices: Ground beef, raw chicken wings, shrimp, cantaloupe, brie cheese, dry infant formula, chocolate, shell eggs, ground black pepper, peanut butter, and dry pet food
- Environmental surfaces: Stainless steel, sealed concrete, plastic, ceramic tile, and rubber

ISO 16140 Validation

BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

ABI 29/02 – 09/10
[End of validity: refer to certificate at www.afnor-validation.com]

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification

The MicroSEQ® *Salmonella* spp. Detection Kit has been certified "NF Validation". The certification uses the ISO 16140 standard for the validation of alternative methods (Alternative Analytical Methods for Agribusiness. Certified by NF Validation; www.afnor-validation.com). This kit was compared and found equivalent to the ISO 6579 reference method. The validated workflow includes:

- The PrepSEQ® Rapid Spin Sample Preparation Kit
- The MicroSEQ® *Salmonella* spp. Detection Kit
- The Applied Biosystems 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software v1.1

The MicroSEQ® *Salmonella* spp. Detection Kit has been certified "NF Validation" for any food and feed category. The tested product categories are meat products (processed and unprocessed), dairy products, egg products, seafood, vegetables, and feeding stuffs.

In the context of NF Validation, all samples identified as positive by the MicroSEQ® *Salmonella* spp. method must be confirmed, from the BPW enrichment broth, by performing on of the following tests:

- By performing a second enrichment step in RVS (0.1 mL BPW in 10 mL of RVS broth): incubate at 41.5 ±1 °C for 24 ±3 h and streak onto XLD agar or another selective agar plate. Perform a Latex test on the observed characteristic colonies. In the case of a negative Latex test, perform a biochemical gallery on purified colonies characteristic of *Salmonella*. If the confirmatory result remains negative, proceed to a second selective enrichment in MKTTn broth and perform the confirmatory tests described in the CEN ISO standardized methods.
- According to classical tests described in methods standardized by CEN ISO from colonies (including purification step).

- Any other method certified by "NF Validation" that is based on a different principle than the MicroSEQ® *Salmonella* spp. method. It is necessary that the complete protocol of the second validated method be performed entirely, which means that all steps that precede the confirmation step must be common to both methods.

In the event of discordant results (positive with the alternative method, non-confirmed by one of the means described above), the laboratory must follow the necessary steps to guarantee the validity of the obtained result.

General remarks and recommendations:

- Comply with Good Laboratory Practices – GLP (Refer to EN ISO 7218 standard).
- For NF Validation, 25 gram samples were tested.
- We recommend that ISO 6579 and ISO 6887 standards be followed for the preparation of "master suspensions".

Expiration date

For more information about the expiration date of the "NF Validation" certification, please refer to the certificate, ABI 29/02 – 09/10, available on the web site at www.afnor-validation.com.

Operational conditions

Altitude

The Applied Biosystems 7500 Fast, StepOne™, and StepOnePlus™ Real-Time PCR Systems are for indoor use only and for altitudes not exceeding 2000 m (6500 ft) above sea level.

Temperature and humidity requirements

Condition	Acceptable range
Temperature	15 to 30 °C (50 to 90 °F) Maximum change of less than 15 degrees Celsius (59 degrees Fahrenheit) per 24 hours
Humidity	20 to 80% relative humidity, noncondensing

Definitions of terms

This protocol uses the following terms:

- **Amplification** – The process of making copies of, and thereby increasing the amount of a specific DNA sequence.
- **Internal Positive Control (IPC)** – A control in all reaction wells that should always yield amplification. If it does not, a problem with amplification exists. The presence of an inhibitor in the unknown sample is indicated if the IPC signal is significantly reduced.
- **Negative control** – A reaction mixture that lacks a target sequence. It indicates contamination if amplification occurs, or an amplification problem if the IPC signal is reduced or absent. The Pathogen Detection Negative Control is provided in the kit as a negative control. At least one negative control is required for each target assay.
- **Polymerase chain reaction (PCR)** – Technology used to amplify, or increase the amount of a DNA sequence.
- **Positive control** – A control that establishes the expected amplification of a target. The lack of a target signal in a positive control well indicates a pipetting error or a problem with amplification. A positive control is provided by the investigator and is recommended but not required for each run.
- **Primer** – A segment of DNA that is complementary to the target DNA sequence or IPC DNA sequence. It is needed to start amplification.
- **Probe** – A segment of DNA that is complementary to the target DNA sequence or IPC DNA sequence. The probe is labeled with a reporter dye. When the probe binds to the target or IPC during the amplification step, fluorescence is emitted. The Sequence Detection System (SDS) or Real-Time PCR System detects the fluorescence, indicating the presence of the target or IPC DNA sequence.
- **Target** – The bacteria being tested.
- **Unknown sample** – A DNA sample from a food substance that you test for the presence of one or more food pathogens.

Materials and equipment

Kit contents

The MicroSEQ® *Salmonella* spp. Detection Kit (PN 4403930) contains reagents for 96 reactions. Kit components and their storage conditions are shown in the table below.

Item	Cap color	Description	Part number	Storage
MicroSEQ® *Salmonella* spp. Detection Kit (Part 1 of 2)	Green (rack) 	*Salmonella* spp. Assay Beads, 96 tubes in 8-tube strips (96 reactions/kit), twelve 8-tube strips	4403870	5 ±3 °C; protect from light
	N/A	MicroAmp® Optical 8-Cap Strips, twelve 8-cap strips		
Pathogen Detection Negative Control (Part 2 of 2)	Red 	Pathogen Detection Negative Control, 1 tube, 1.5-mL (PN 4403926)	4403927	5 ±3 °C

Storage

- When you receive the Assay Beads and Pathogen Detection Negative Control, store them at 5 ±3 °C. Protect from light. Excessive exposure to light may affect the fluorescent probes.
- Seal the pouch tightly each time you remove 8-tube strips from the pouch to protect from moisture.

Shelf life

The kit expires 18 months after manufacture.

Materials not included in the kit

The following table includes materials that are required for using (but not included in) the MicroSEQ® *Salmonella* spp. Detection Kit (PN 4403930). Unless otherwise indicated, many of the listed items are available from major laboratory suppliers (MLS).

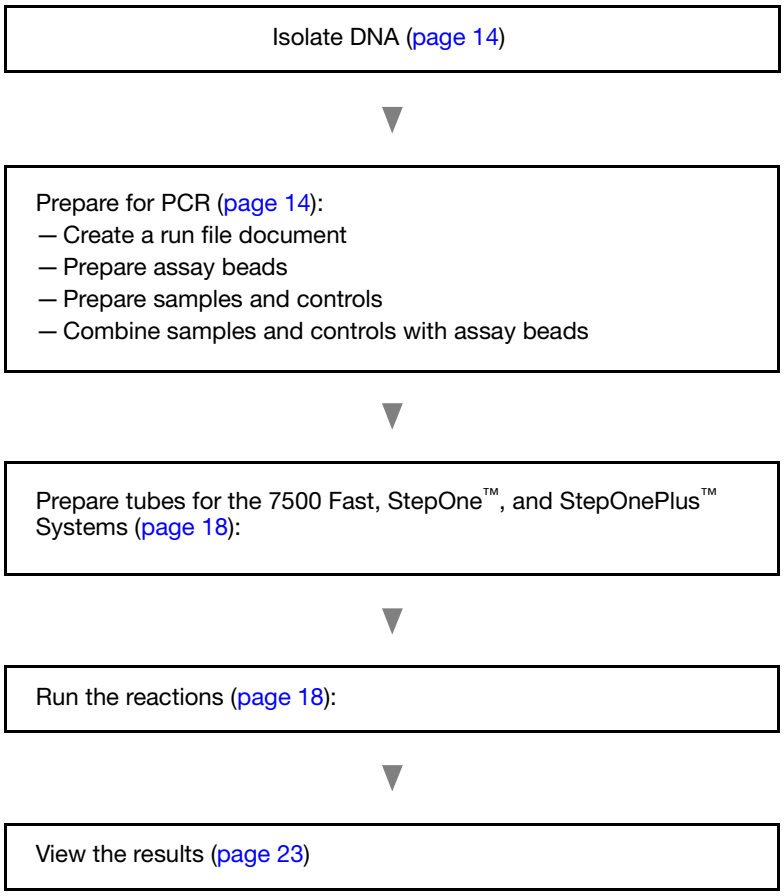
Instruments, equipment, consumables, and reagents

Item	Source
Instruments	
Applied Biosystems 7500 Fast Real-Time PCR System	Contact your local Applied Biosystems sales office.
StepOne™ Real-Time PCR System	
StepOnePlus™ Real-Time PCR System	
Equipment	
Benchtop microcentrifuge	MLS
Block heater	MLS
Ice bucket	MLS
Plate centrifuge	MLS
Vortexer	MLS
7500 Fast Precision Plate Holder for MicroAmp® Tube Strips (for use with 7500 Fast Real-Time PCR System‡)	Applied Biosystems PN 4403809
MicroAmp® Fast 48-Well Tray (for use with StepOne Real-Time PCR System)	Applied Biosystems PN 4375282
MicroAmp® 96-Well Tray for Veriflex™ Blocks (for use with StepOnePlus Real-Time PCR System)	Applied Biosystems PN 4379983
Consumables	
Aerosol-resistant pipette tips	MLS
Disposable gloves	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
MicroAmp® Fast 8-Tube Strip, 0.1-mL	Applied Biosystems PN 4358293
MicroAmp® Optical 8-Cap Strip, 300 strips	Applied Biosystems PN 4323032
Reagents	
DNase-free, sterile-filtered water	MLS

‡ included in the starter kit

Kit workflow

The MicroSEQ® *Salmonella* spp. Detection Kit workflow is shown below.



Isolate DNA

The PrepSEQ® Nucleic Acid Extraction Kit Protocol: *Salmonella* spp. (PN 4405968; magnetic-bead-based) or PrepSEQ® Rapid Spin Sample Preparation Kit Protocol: *Salmonella* spp. (PN 4412848; spin-column-based) is recommended when you use the MicroSEQ® *Salmonella* spp. Detection Kit.

Prepare for PCR

To prepare for PCR, you must create a run file document and prepare the assay beads and samples. The exact procedure depends on whether or not you use RapidFinder™ Express Software. This protocol describes the procedure that does not use RapidFinder Express Software. We recommend using RapidFinder Express Software for online step-by-step instructions to set up the assays followed by automated data analysis. For details, refer to the *RapidFinder™ Express Software Online Help* (PN 4401842).

IMPORTANT! If you use RapidFinder™ Express Software, refer to the "Create or Edit Run File" and "Pipette Samples" instructions in the *RapidFinder™ Express Software Online Help* (PN 4401842).

Prepare PCR (without RapidFinder™ Express software)

Create a run file document

1. In the Sequence Detection System Software, create a run file document: In the Assay drop-down list, select **Absolute Quantification** (**Standard Curve**). For information on creating a run file document, refer to the documentation that is provided with your instrument.
2. With the Quencher Dye set to (**none**) or (**Non Fluorescent**), create or select FAM™ and VIC® dye detectors.
3. Associate both FAM™ and VIC® detectors with each reaction.

   Note: The FAM dye is used to detect the target; the VIC dye is used to detect the internal positive control (IPC).

4. Set thermal-cycling conditions as indicated in the table below. For more details, refer to the *7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide* or the *StepOne™ and StepOnePlus™ Real-Time PCR Systems Presence/Absence Experiments Getting Started Guide*.

For 7500 Fast instruments

Step	Enzyme activation	PCR	
	HOLD	Cycle (40 cycles)	
		Denature	Anneal/extend
Temp.	95 °C	95 °C	60 °C
Time	2 min	3 sec	30 sec

For StepOne and StepOnePlus instruments
(not included in AOAC, nor AFNOR validation studies)

Step	Enzyme activation	PCR	
	HOLD	Cycle (40 cycles)	
		Denature	Anneal/extend
Temp.	95 °C	95 °C	60 °C
Time	2 min	1 sec	20 sec

5. Set the Sample Volume to **30** µL.

6. Select the appropriate run mode for your system according to the following table:

System	Run mode
7500 Fast‡	Fast 7500
StepOne	Fast
StepOnePlus	Fast

‡ Figure 1 shows an example of the Instrument tab of the 7500 Fast System SDS Software

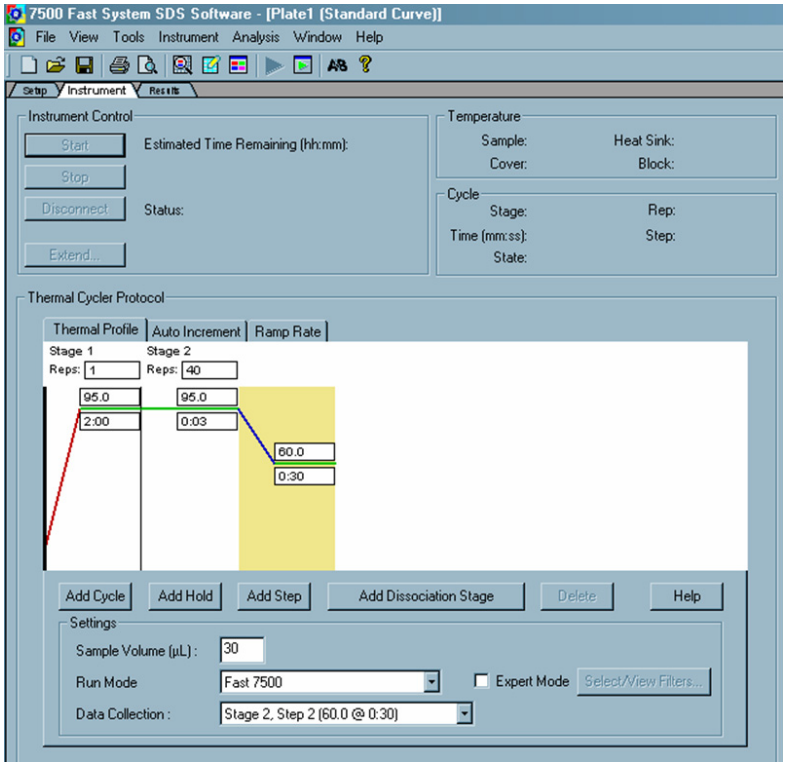


Figure 1 Instrument tab for 7500 Fast System SDS Software with run mode set to Fast 7500

Prepare the assay beads

WARNING! CHEMICAL HAZARD. MicroSEQ® *Salmonella* spp. Detection Kit (Part 1 of 2).

CAUTION! CHEMICAL HAZARD. Pathogen Detection Negative Control (Part 2 of 2).

1. Open the storage pouch containing the assay beads (cut at the notch in the upper corner of the storage pouch above the zip-lock strip).

   IMPORTANT! Do not remove the desiccant from the storage pouch.

2. Remove the appropriate number of individual tubes or 8-tube strips, one tube for each reaction that you plan to run.

3. Seal the storage pouch using the zip-lock strip, then store at 5 ±3 °C.

Prepare samples and controls

1. Thaw all reagents (samples and controls) completely.
2. Remove condensation after thawing samples and prior to opening to avoid cross contamination.
   a. For PrepSEQ Nucleic Acid Extraction Kit samples, centrifuge the plate at 2000 × g for 1 minute.
   b. For PrepSEQ Rapid Spin Sample Preparation Kit samples, microcentrifuge the tubes for 1 minute at maximum speed to bring down any particulate material derived from the spin column, which can interfere with amplification. The microbial DNA is in the aqueous phase.
   c. For Pathogen Detection Negative Control and positive control(s) in microcentrifuge tubes, vortex and then spin down the tubes.

Note: Unknown samples and positive control samples are provided by the investigator. The kit includes a negative control (Pathogen Detection Negative Control).

3. Add 30 µL of sample prepared above to each assay bead. Dispense all unknown samples first, followed by negative control(s), and then positive control(s). Beads dissolve in 1 to 5 seconds. Mix by gently aspirating and dispensing a few times. (Alternatively, vortex the assay tubes after they are capped in the final step.)

IMPORTANT! Use a new pipette tip for each sample. Resuspend by gently pipetting up and down with the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination or, if a vortexer is available, vortex the capped tubes until the pellet is dissolved.

Note: Add 30 µL of the PrepSEQ Rapid Spin Kit sample, or 30 µL of the PrepSEQ Nucleic Acid Extraction Kit sample, to each assay bead. The PrepSEQ Rapid Spin Kit provides 300 µL of sample, and the PrepSEQ Nucleic Acid Extraction Kit provides ~100 µL of sample. Use 30 µL of the Pathogen Detection Negative Control per negative control reaction. If you use other sample preparation methods, and less than 30 µL of sample is available, adjust the sample volume with water to 30 µL for each reaction type.

IMPORTANT! Cap the tubes, sealing each tube with the transparent optical strip caps provided in the kit. Do not use colored caps or tubes for kit reactions. Colored caps or tubes may affect dye-signal readings during real-time PCR.

Note: The assay beads contain all the components necessary for each reaction.

Prepare tubes for the 7500 Fast, StepOne™, and StepOnePlus™ Systems

8-tube strips containing assay beads are compatible with 7500 Fast, StepOne™, and StepOnePlus™ Systems.

1. For 8-tube strips with seven or fewer reactions, add additional empty tubes as needed so that each strip contains a full set of 8 tubes.

   Note: The empty capped 8-tube strips evenly distribute the clamping load that is applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes. Add empty tubes as needed.

2. Cap the tubes, sealing each tube with the transparent optical strip caps provided in the kit. Cap the tubes with the strip caps using the MicroAmp® 96-Well Base (PN N8010531) and the MicroAmp® Cap Installing Tool (Handle, PN 4330015) to avoid collapsing, bending, or misaligning the tubes. Confirm that the strips are straight and that each tube is in line with the adjacent tube.

3. Make sure reactions are thoroughly mixed.

   IMPORTANT! Mixing can be accomplished by gentle pipetting up and down. Keep the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination, or, if available, use a vortexer to mix the assay tube components.

4. Make sure reagents are at the bottom of the tubes. If available, spin down the tube contents at 2000 × g for 20 seconds using a centrifuge with a plate adapter.

   Proceed to "Run the reactions" on page 18.

Run the reactions

Running tubes or a plate involves using an Applied Biosystems Sequence Detection System (SDS) or Real-Time PCR System. The exact procedure depends on whether or not you use RapidFinder™ Express Software. This protocol describes the procedure that does not use RapidFinder Express Software. We recommend using RapidFinder Express Software for online step-by-step instructions to set up the assays followed by automated data analysis. For details, refer to the *RapidFinder™ Express Software Online Help* (PN 4401842).

IMPORTANT! If you use RapidFinder™ Express Software, refer to the "Start Instrument Run" instructions in the *RapidFinder™ Express Software Online Help* (PN 4401842).

Before you begin

Ensure that your instrument is properly installed and calibrated. For calibration information, see the documentation that is provided with your instrument.

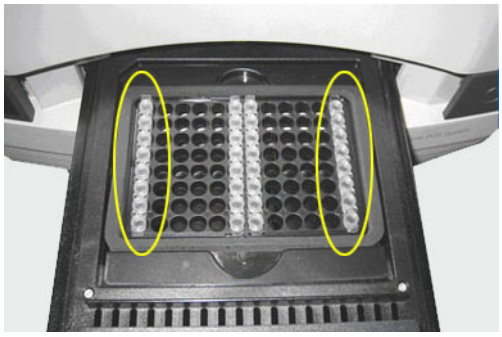
Run the 8-tube reactions on the 7500 Fast System (only) without RapidFinder™ Express Software

For 8-tube strips on the 7500 Fast System:

Use of the 7500 Fast Precision Plate Holder for MicroAmp® Tube Strips (PN 4403809) is recommended.

When you use the MicroAmp 8-tube strips, if column 1 (leftmost) and column 12 (rightmost) are not used, insert two fully capped, empty, 8-tube strips into these columns.

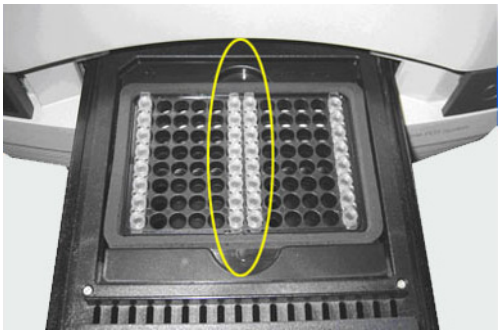
Note: The empty capped 8-tube strips evenly distribute the clamping load applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes.



1. Carefully insert two or more 8-tube strips containing samples, starting from the center of the plate holder and moving out. This layout minimizes bending or misaligning the tube strips.

   Note: A minimum of two and a maximum of twelve 8-tube strips can be run at one time. If your samples are in an odd number of strip tubes, you will need to include an empty capped 8-tube strip to balance the load.

   IMPORTANT! Always use a total of 8 tubes per column. You may need to add new, empty tubes to a column.

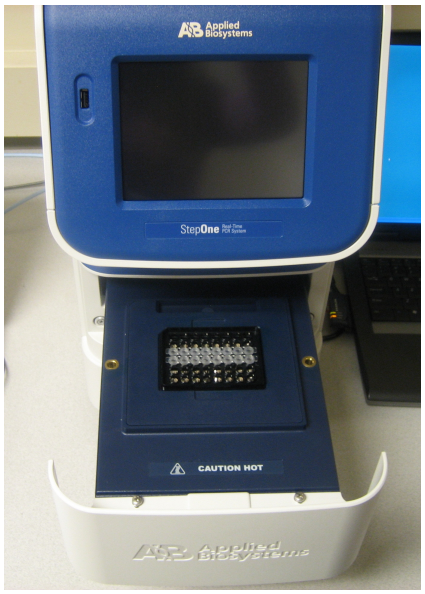


2. Open the run file document that corresponds to the reaction plate that you created in "Create a run file document" on page 14.

3. Start the run.

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

Run the 8-tube reactions on the StepOne™ System



1. Place the MicroAmp® Fast 48-Well Tray (PN 4375282) on the sample block of the StepOne System.

2. Load the 8-tube strips horizontally. For example, in Row C, load an 8-tube strip across columns 1 through 8. A minimum of one 8-tube strip is recommended. It is not necessary to balance the tube strips on the tray.

3. Open the run file document that corresponds to the reaction plate that you created in "Create a run file document" on page 14.

4. Start the run.

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

Run the 8-tube reactions on the StepOnePlus™ System

1. Place the MicroAmp® 96-Well Tray for Veriflex™ Blocks (PN 4379983) on the sample block of the StepOnePlus System.

2. Load the 8-tube strips vertically. For example, load the 8-tube strips in columns 1 and 2 across rows A through H in both columns. The minimum recommended load is two 8-tube strips (16 tubes), which you should place in adjacent columns, for example in columns 1 and 2. It is not necessary to balance the tube strips on the tray.

3. Open the run file document that corresponds to the reaction plate that you created in "Create a run file document" on page 14.

4. Start the run.

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

View results

Overview

How you view results depends on the instrument you use. Refer to the appropriate user guide of your real-time PCR instrument or the *RapidFinder™ Express Software Online Help* for instructions on how to analyze data and view your results.

IMPORTANT! If you use RapidFinder™ Express Software, refer to the "Viewing Results" instructions in the *RapidFinder™ Express Software Online Help* (PN 4401842).

General process without RapidFinder™ Express Software

The general process for viewing results from MicroSEQ® Pathogen Detection Kits involves:

- Viewing the amplification plots for all reactions.
- Setting the baseline and threshold values.
- Checking each sample for a FAM™ dye (target-specific) signal and a VIC® dye (IPC) signal. The following table is a basic guide for interpreting the results:

FAM dye signal (target)	VIC dye signal (IPC)	Result
+	+, –	Positive
–	+	Negative
–	–	See "Troubleshooting" on page 24

Resources for viewing results

For more information about analyzing your data, refer to the:

- Appropriate instrument user guide
- *RapidFinder™ Express Software Online Help*

Applied Biosystems does not recommend using the same method to screen samples and to confirm the results. When you use the MicroSEQ® Pathogen Detection System to screen samples, culture and biochemical methods are recommended to confirm the result. ISO 6579:2002 (E), (see "References" on page 25) is an approved protocol for confirming culture testing.

The confirmatory methods that can be used to confirm positive results in the context of NF Validation are described on pages 8 and 9.

Troubleshooting

Observation	Possible cause	Action
No IPC or target-specific signal is detected in unknown wells.	Inhibition of PCR occurred.	Dilute the sample 1:5 or 1:10 with water in order to dilute PCR inhibitors and repeat the assay. If PCR remains inhibited, repeat the sample preparation. For more information, refer to "Troubleshooting" section of the PrepSEQ Nucleic Acid Extraction Kit Protocol: *Salmonella* spp. (PN 4405968), PrepSEQ Rapid Spin Sample Preparation Kit Protocol: *Salmonella* spp. (PN 4412848).
No target-specific signal is detected in positive control wells.	A pipetting error occurred (no positive control was added).	Repeat the assay. Make sure to pipette positive control into all positive-control wells.
No IPC is detected, but target-specific signal is detected.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.	No action is required.
Target-specific signal is detected in negative-control wells	Carryover contamination occurred.	Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. If the negative control continues to show contamination, repeat the assay using a new kit. If the negative control continues to show contamination, contact Applied Biosystems Technical Support.
In negative-control wells, a target-specific signal is detected, but no IPC signal is detected.	Carryover contamination and one of the following occurred: • A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA • A problem occurred with IPC amplification	Examine unknowns to determine if an IPC signal is present. If an IPC signal is present in unknown wells, IPC amplification is not a problem. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.
No IPC signal is detected, but target-specific signal is detected in positive-control wells.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.	No action is required.
Replicate results for this sample are inconsistent.	All replicate wells for a sample do not have the same result.	If more than two replicates yield the same result (for example, you ran 3 replicates and 2 replicates are negative, but 1 replicate is positive), it is probable that the result with the larger number of replicates is accurate. However, your laboratory protocol may require that you repeat the assay using fresh samples and reagents. If you ran only two replicates and results are not consistent, repeat the assay using fresh samples and reagents.

References

Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. Reference number ISO 6579:2002 (E).

Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Reference number ISO 6887, parts 1 through 4.

Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations. Reference number ISO 7218:2007.

Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods. Reference number ISO 16140:2003.

2010. Association of Analytical Chemists (AOAC). http://www.aoac.org. Accessed November 2010.

2010. Association Francaise pour la Normalisation (AFNOR). http://www.afnor-validation.com. Accessed November 2010.

Good PCR Practices

Prevent contamination and nonspecific amplification

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.

PCR good laboratory practices

When preparing samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution.

IMPORTANT! To avoid false positives due to cross-contamination:

- Prepare and close all negative-control and unknown sample tubes before pipetting the positive control.
- Do not open tubes after amplification.
- Use different sets of pipettors when pipetting negative-control, unknown, and positive-control samples.

Appendix B

Safety

This appendix covers:

Chemical safety

Chemical hazard warning

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

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.

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.

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 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 31.)
- 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.

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**, click **Support**, then select **SDS**.
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 select

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

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets (SDSs) 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.

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

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 published in *Biosafety in Microbiological and Biomedical Laboratories* (**http://www.cdc.gov/biosafety/publications/index.htm**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **http://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: **http://www.cdc.gov**

Chemical alerts

General alerts for all chemicals

Avoid contact with (skin, eyes, and/or clothing). Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. MicroSEQ® *Salmonella* spp. Detection Kit (Part 1 of 2). Harmful if swallowed or inhaled. Causes eye, skin, and respiratory tract irritation. Do not taste or swallow. Avoid breathing vapor. Read the SDS and follow the handling instructions. Use with adequate ventilation. Avoid contact with eyes and skin. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION! CHEMICAL HAZARD. Pathogen Detection Negative Control (Part 2 of 2). Some reagents may contain sodium azide. It can react with lead and copper plumbing to form explosive metal azides. Dispose of waste in accordance with all applicable regulations**.**

Related documentation

For additional documentation, see "How to obtain support" on page 6.

For information on new assays and updated product documentation, go to **http://info.appliedbiosystems.com/pathogenkits**

Real-time PCR system	Document title	PN
All real-time PCR systems	*MicroSEQ® Salmonella spp. Detection Kit Quick Reference Card*	4405963
	MicroSEQ® Listeria monocytogenes Detection Kit Protocol	4405962
	MicroSEQ® Listeria monocytogenes Detection Kit Quick Reference Card	4405961
	PrepSEQ® Nucleic Acid Extraction Kit Protocol	4400739
	PrepSEQ® Nucleic Acid Extraction Kit Quick Reference Card	4406303
	PrepSEQ® Nucleic Acid Extraction Kit Protocol: Salmonella spp.	4405968
	PrepSEQ® Nucleic Acid Extraction Kit Quick Reference Card: Salmonella spp.	4405967
	PrepSEQ® Nucleic Acid Extraction Kit Protocol: Listeria monocytogenes	4405966
	PrepSEQ® Nucleic Acid Extraction Kit Quick Reference Card: Listeria monocytogenes	4405965
	PrepSEQ® Rapid Spin Sample Preparation Kit Protocol	4412847
	PrepSEQ® Rapid Spin Sample Preparation Kit Quick Reference Card	4412846
	PrepSEQ® Rapid Spin Sample Preparation Kit Protocol: Salmonella spp.	4412848
	PrepSEQ® Rapid Spin Sample Preparation Kit Quick Reference Card: Salmonella spp.	4412849
	PrepSEQ® Rapid Spin Sample Preparation Kit Protocol: Listeria monocytogenes	4412851
	PrepSEQ® Rapid Spin Sample Preparation Kit Quick Reference Card: Listeria monocytogenes	4412852
	Introduction to TaqMan® and SYBR® Green Chemistries for Real-Time PCR Protocol	4407003
RapidFinder Express Software	*RapidFinder™ Express v1.0 Online Help*	4401842
	RapidFinder™ Express v1.0 Quick Reference Card	4410241
	RapidFinder™ Express Software v1.0 and SDS Software v1.4.1 User Bulletin	4407982
7500 Fast System	*Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide*	4347825
StepOne™ and StepOnePlus™ Systems	*Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Presence/Absence Experiments Getting Started Guide*	4376787

Portable document format (PDF) versions of this guide and the documents listed above are available at **www.appliedbiosystems.com**

Note: To open the documentation available from the Applied Biosystems web site, use the Adobe® Acrobat® Reader® software available at **www.adobe.com**

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