

RapidFinder™ *Salmonella* species, *Typhimurium* and *Enteritidis* Multiplex PCR Kits

USER GUIDE

Direct lysis and real-time PCR detection of *Salmonella* species,
S. Enteritidis, and *S. Typhimurium* in food and environmental
samples

for use with:

Applied Biosystems™ 7500 Fast Real-Time PCR Instrument with Applied
Biosystems™ RapidFinder™ Express Software v2.0 or later

Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument with Thermo
Scientific™ RapidFinder™ Analysis Software v2.0 or later

KingFisher™ Flex Purification System with 96 Deep-Well Head

Catalog Numbers A33227, A33227KF

Publication Number MAN0015918

Revision D.0



For testing of Food and Environmental samples only.

ThermoFisher
S C I E N T I F I C

Revision history: Pub. No. MAN0015918

Revision	Date	Description
D.0	11 May 2023	<ul style="list-style-type: none">• The RFE assay file name was updated.• The RFA software version was updated.• A second enrichment step was added for shell eggs.
C.0	15 January 2021	<ul style="list-style-type: none">• The software versions were updated.• Minced turkey was changed to ground turkey.
B.0	1 March 2019	Three matrices were added—shell eggs, poultry carcasses, and minced turkey.
A.0	11 October 2017	New document for the RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kits

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Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

Name and intended use

The Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kits enable real-time PCR detection and differentiation of *Salmonella* species, *Salmonella* ser. Typhimurium, and *Salmonella* ser. Enteritidis from meat, production environment samples, and primary production samples (see Table 19). These kits are intended for use in laboratories undertaking microbiological analysis, and they are compatible with the following instruments and software:

PCR instrument	Software	Pathogen Assay File
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument	Thermo Scientific™ RapidFinder™ Analysis Software v2.0 or later	RapidFinder Salmonella SP-SE-ST Multiplex version 2.0 or later ^[1]
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument	Applied Biosystems™ RapidFinder™ Express Software v2.0 or later	ST_Multiplex_RapidFinder_7500_2.0 or later ^[1]

^[1] Assay files and instructions are available at thermofisher.com/molecular-microbiology-software.

Use the kit appropriate for the sample matrix:

Sample matrix ^[1]	Kit	Description
<ul style="list-style-type: none"> 25-g food (poultry and pork meat) Production environment samples <ul style="list-style-type: none"> Carcass rinse Shell eggs 	RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (Cat. No. A33227)	<ul style="list-style-type: none"> Reagents for bacterial cell lysis and release of DNA from bacteria Lyophilized target-specific primers, probes, and reagents for multiplex real-time PCR using the prepared lysate
375 g Ground turkey	RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex Flex Kit (Cat. No. A33227KF)	<ul style="list-style-type: none"> Reagents for selective capture and concentration of <i>Salmonella</i>, followed by lysis and release of DNA from the captured bacteria Lyophilized target-specific primers, probes, and reagents for multiplex real-time PCR using the prepared lysate

^[1] See Table 19 for details on validated matrices.

Principle of the test

The assay is based on TaqMan™ real-time PCR technology. Dye-labeled probes target unique DNA sequences specific to *Salmonella* ser. Typhimurium, *Salmonella* ser. Enteritidis and all *Salmonella* species, and an internal positive control (IPC). Target DNA, if present, is amplified by PCR and detected in real-time using fluorescent hydrolysis probe chemistry. The fluorescence signal that is generated is detected by the real-time PCR instrument and interpreted by the analysis software. For more information about real-time PCR, go to thermofisher.com/qpcducation.

The IPC template, primers, and probe provide an internal control with each reaction that the PCR process has occurred. It is unnecessary to incorporate positive control organisms with routine testing of samples.

Procedure overview

For 25-g food, 30 mL carcass rinses, up to 20 shell eggs, and production environment samples, enriched samples are combined directly with Lysis Reagent 1 and Proteinase K, to lyse any bacterial cells present in the sample and release their DNA into solution.

For 375-g ground turkey samples, enriched samples undergo selective capture and concentration of *Salmonella*, using Dynabeads™ anti-Salmonella and automated immunomagnetic separation with the KingFisher™ Flex Purification System with 96 Deep-Well Head. The purified samples are eluted directly into Lysis Reagent 1 and Proteinase K for lysis.

Lysates are transferred to the Salmonella Multiplex (Sp/SE/ST) PCR Tubes to rehydrate the lyophilized PCR pellet. The PCR tubes are sealed, loaded into the real-time PCR instrument, and the run is started using the RapidFinder™ software. After the run is complete, the software displays the interpreted results as simple positive or negative symbols. The results can be reported, stored, printed, and downloaded as required.

Results are achieved approximately 50 minutes after loading the prepared sample into the instrument.

Limitations

- The test is designed to detect DNA from target organisms that are present at a minimum level of 1 CFU/sample, and have grown to detectable levels during the enrichment.
- The customer is responsible for validation of sample matrices or culture media not described in this document.
- When testing a sample type or culture medium that has not been validated, we recommend testing a selection of known negative and positive samples, to ensure that expected results are achieved. See “Test control organisms” on page 38 and EN ISO 22174:2005.
- See Appendix A, “Troubleshooting” for additional information.

Contents and storage

Store the unopened kits at 2–8°C. Store the individual components as indicated in the following tables.

Table 1 RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit, 96 tests (Cat. No. A33227)

Contents	Amount	Storage ^[1]
Lysis Reagent 1 Tubes (clear, pale blue liquid containing fine white particles) ^[2]	12 strips of 8 tubes ^[3] 170 µL each	2–8°C ^[4] Protect from light
Lysis Tube Caps ^[2]	12 strips of 8 caps ^[3]	Room temperature
Proteinase K (clear colorless liquid)	1.2 mL	2–8°C
Salmonella Multiplex (Sp/SE/ST) PCR Tubes	12 strips of 8 tubes ^[3] 1 pellet each	2–8°C ^[4] Protect from light
PCR Caps	12 strips of 8 caps ^[3]	Room temperature

^[1] See the label for the expiration date. Do not use the reagents after the expiration date indicated on the label.

^[2] Not used for 375-g minced turkey.

^[3] Strips can be cut to select the required number for each run.

^[4] Bring to room temperature before opening.

Table 2 RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex Flex Kit, 96 tests (Cat. No. A33227KF)

Contents	Amount	Storage ^[1]
Dynabeads™ anti-Salmonella	5 mL	2–8°C ^[2]
Lysis Reagent 1 (clear, pale blue liquid containing fine white particles)	2 × 14 mL	2–8°C ^[2] Protect from light.
Proteinase K (clear colorless liquid)	2 × 1.2 mL	2–8°C
Salmonella Multiplex (Sp/SE/ST) PCR Tubes	12 strips of 8 tubes ^[3] 1 pellet each	2–8°C ^[2] Protect from light.
PCR Caps	12 strips of 8 caps ^[3]	Room temperature

^[1] See the label for the expiration date. Do not use the reagents after the expiration date indicated on the label.

^[2] Bring to room temperature before opening.

^[3] Strips can be cut to select the required number for each run.

Required materials

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or [thermofisher.com](https://www.thermofisher.com). They may also be available through Fisher Scientific ([fisherscientific.com](https://www.fisherscientific.com)), MLS, or another major laboratory supplier.

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

Materials for enrichment and sampling

Table 3 Materials for enrichment and sampling

Item	Source
Homogenizer laboratory blender or diluter, one of the following or equivalent:	
<ul style="list-style-type: none"> Homogenizer Laboratory Blender Diluflux™ Pro Automated Gravimetric Dilutor with simple (non-robotic) dispensing arm Diluflux™ Pro Automated Gravimetric Dilutor with robotic dispensing arm 	DB5000A DB4100A DB4150A
Sample enrichment bags, one of the following or equivalent:	
<ul style="list-style-type: none"> BagFilter™ 400 (400 mL) BagPage™ 400 (400 mL) BagLight™ 400 (400 mL) RollBag™ 1300 (1300 mL) 	DB4011A DB4012A DB4013A DB4014A
Incubators fitted with racks for homogenizer bags	thermofisher.com
Buffered Peptone Water (ISO) ^[1]	CM1211B or equivalent performing
Oxoid™ Novobiocin Selective Supplement ^[1]	SR0181E , SR0249A , or equivalent
Modified Tryptone Soya Broth (Dehydrated)	CM0989

^[1] Not required for enrichment of primary production samples.

Table 4 Additional materials for enrichment of production environment samples

Item	Source
Dey-Engley Broth or other neutralizing broth, or Peptone Water, as appropriate for the sample type	MLS
Sterile sampling swabs, wipes, or sponges, for example: Remel™ bio-spo Sponge	<ul style="list-style-type: none"> Sponges: R658003 or equivalent Swabs, wipes: MLS

Table 5 Additional materials for enrichment of shell eggs and 375-g minced turkey samples

Item	Source
Large Homogenizer Laboratory Blender	MLS
Large homogenizer bags appropriate for the sample type and size	MLS

Materials for lysis

Table 6 Materials for lysis of enriched cultures

Item	Source
Applied Biosystems™ SimpliAmp™ Thermal Cycler	A24811
MicroAmp™ 96-Well Tray/Retainer Set for Veriti™ Systems ^[1]	4381850
MicroAmp™ Splash-Free 96-Well Base	4312063
Sample pipette, 1- to 10-mL	4642110 or equivalent
Adjustable single- or multi-channel pipettor, 5- to 50-µL	4642130 (single), 4662010 (multi-channel) or equivalent
(Optional but recommended) Repeat pipettor, 10- to 100-µL	46200400 or equivalent
Compact PCR tube rack, mixed colors	Available through the Thermo Fisher Microbiology ordering process.
Tool for capping and decapping	
Extra-long filtered pipette tips, 1- to 10-mL	MLS
Pipette tips, aerosol resistant	94052100 (10 µL) or equivalent
Microcentrifuge tubes, 1.5-mL	MLS

^[1] We recommend using the tray only from the MicroAmp™ 96-Well Tray/Retainer Set for Veriti™ Systems. If you are not using the tray/retainer set, then tube strips are required for balancing the SimpliAmp™ Thermal Cycler. To prevent crushing of tubes, include at least 4 complete tube strips, placed evenly across the heat block.

Table 7 Additional materials for lysis of enriched 375-g minced turkey samples

Item	Source
Equipment	
KingFisher™ Flex Purification System with 96 Deep-Well Head ^[1]	A32681 or equivalent (contact your local Thermo Scientific Microbiology sales representative)
KingFisher™ Deep-Well 96 Plate, V-bottom	95040450
KingFisher™ Flex 96 Deep-Well Heating Block	24075430
KingFisher™ 96 tip comb for DW magnets	97002534
Multichannel pipettes; choose manual or electronic:	
Finnpipette™ F2 Multichannel Pipettes, manual	4662020 (10–100 µL) or equivalent
(Optional but recommended) Finnpipette™ Novus Electronic Multichannel Pipettes	46300400 (30–300 µL) or equivalent 46300800 (100–1200 µL) or equivalent
Plastics, consumables, and reagents	
Pipette tips, aerosol resistant	94052410 (1000 µL) or equivalent
Adhesive Plate Seals, pack of 100	AB-0580
Oxoid™ Phosphate Buffered Saline Tablets ^[2]	BR0014G
Tween™ 20 ^[2]	MLS

^[1] Operation of the KingFisher™ Flex Magnetic Particle Processor can also be controlled with BindIt™ Software (included) run on an external computer.

^[2] Required to make Wash Buffer, see “Prepare the Wash Buffer” on page 25.

Materials for PCR

Table 8 Materials for PCR

Item	Source
Real-time PCR instrument and accessories, one of the following instrument packages	
QuantStudio™ 5 Real-Time PCR Instrument, 0.1-mL block, with RapidFinder™ Analysis Software v2.0 or later	A36320 (desktop) A36328 (laptop) Contact your local microbiology sales representative
7500 Fast Real-Time PCR Instrument with RapidFinder™ Express Software v2.0 or later	A30304 (desktop) A30299 (laptop) Contact your local microbiology sales representative
For the QuantStudio™ 5 Food Safety Real-Time PCR Instrument	
MicroAmp™ 96-Well Tray for VeriFlex™ Block	4379983

Table 8 Materials for PCR (continued)

Item	Source
MicroAmp™ Splash-Free 96-Well Base	4312063
For the 7500 Fast Food Safety Real-Time PCR Instrument	
PCR Carry plate for SureTect™ assays, and one of the following plate holders with accessories:	PT0695
Precision Plate holder for SureTect™ assays	PT0690
Low Profile Tubes, strips of 8, white ^[1]	AB0771W
Ultra Clear qPCR Caps, strips of 8 ^[1]	AB0866
7500 Fast Precision Plate Holder, for 0.1 mL tube strips	A29252
MicroAmp™ Fast 8-Tube Strip, 0.1 mL ^[2]	4358293
MicroAmp™ Optical 8-Cap Strips ^[2]	4323032
Additional materials for PCR	
Vortex mixer	Available through the Thermo Fisher Microbiology ordering process. See thermofisher.com/plastics for more information.
8-channel pipette, 10- to 100-µL	
Filtered pipette tips, 10- to 100-µL	

^[1] Used for balancing.^[2] Required to balance the lid pressure if less than 2 full strips are processed.

Materials for confirmation testing

Table 9 Materials for confirmation of positive results

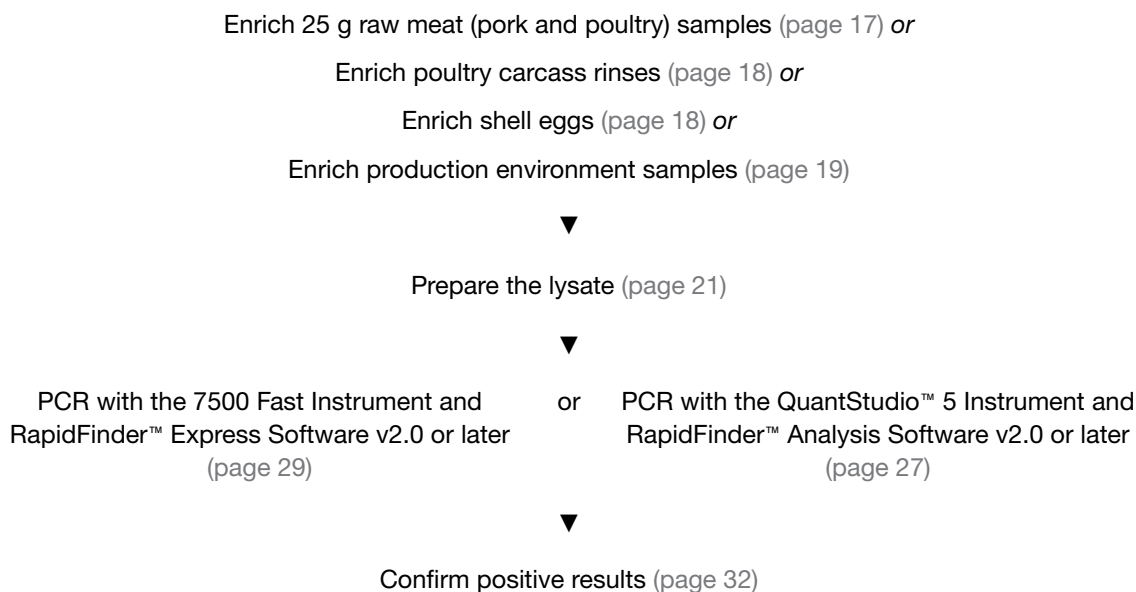
Item	Source
Oxoid™ <i>Brilliance</i> ™ Salmonella Agar	North America: <ul style="list-style-type: none"> • R110374 • CM1092B (base) • SR0194E (supplement)^[1] Outside North America: <ul style="list-style-type: none"> • PO5098A
Saline	MLS
Remel™ Nutrient Agar	<ul style="list-style-type: none"> • North America: R061570 • Outside North America: BO0336E
Oxoid™ Salmonella Test Kit (latex test)	DR1108A

Table 9 Materials for confirmation of positive results *(continued)*

Item	Source
Remel™ Agglutinating Sera for serotype confirmation (or equivalent)	
Salmonella O Factor 4 (Group B)	R30956901
Salmonella H (i)	R30161601
Salmonella H (1,2)	R30163301
Salmonella O Factor 9 (Group D)	R30957301
Salmonella H (g,m)	MLS
Salmonella H (1,7)	MLS

[1] Contact your Thermo Scientific Microbiology sales representative for prepared media options.

Workflow





Procedural guidelines

Guidelines for sample enrichment

- For preparation of master suspensions, follow the instructions of EN ISO 6887 and EN ISO 6579 standards. Comply with Good Laboratory Practices (refer to EN ISO 7218 standard).
- Follow the manufacturer's instructions for preparation of culture media.
- For consistent PCR results:
 - Use a ventilated incubator.
 - Follow the specified temperature and time allowances.
- Dispose of all inoculated culture media as hazardous microbiological waste, even if shown to be negative for the target organism, according to local guidelines.

Guidelines for sample lysis

- Follow the specified temperature allowances.
- For downstream PCR on the **7500 Fast instrument or the QuantStudio™ 5 Instrument** — Prepare a mock-purified sample using sterile enrichment media as a negative extraction control. (The negative extraction control is required for RapidFinder™ Express Software; it is optional but recommended for RapidFinder™ Analysis Software.)
Add the enriched sample or negative extraction control to the bottom of the lysis tube.
- For the **thermal cycler method** — To prevent crushing tubes, use the tray *only* from the MicroAmp™ 96-Well Tray/Retainer Set provided with the SimpliAmp™ Thermal Cycler. See the *SimpliAmp™ Thermal Cycler User Guide* (Pub. No. MAN0009889). Alternatively, use at least 4 complete tube strips in the heat block. We recommend spacing the strips evenly across the heat block. If needed, add empty SureTect™ tubes to make 4 complete strips.

Guidelines for PCR

-
- **IMPORTANT!** After the lysate has been added to the pellets, ensure that the pellet rehydrates immediately by tapping the tubes on the lab bench. Start the PCR run within 30 minutes.
-
- Tube and cap strips can be cut when less than a full strip is required.
Do not cut the strips of caps or tubes too close to the wall of the tube or the cap lid, otherwise the lid might not seal adequately during PCR.

- Particulate matter from the lysate can inhibit the PCR. To ensure that no particles are transferred to the PCR tube, remove lysate from the top half of the liquid, taking care not to disrupt the particles at the bottom of the tube.
If the particles become disturbed, allow the particles to resettle for 1–2 minutes before lysate removal.
- Ensure that the pellet is fully dissolved. The solution changes from blue to green when the pellet is dissolved.
- For ease of use, a multi-channel pipettor can be used to transfer multiple lysates to the PCR tubes.
- Follow “Good laboratory practices for PCR” on page 40.



Figure 1 Avoid lysis particles

3

Enrich and prepare lysates from food or environmental samples

Use the RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (Cat. No. A33227) for this procedure.

Enrich 25 g raw meat (pork and poultry) samples

1. Transfer the sample to a homogenizer bag, then add the media, as indicated.

Table 10 Enrichment conditions for raw meat (pork and poultry) samples

Sample size	PCR Instrument	Volume of media/sample	Media	Incubation
25 g raw pork or raw poultry	<ul style="list-style-type: none"> • QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v2.0 or later • 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later 	225 mL	Buffered Peptone Water with 12 mg/L novobiocin	41.5±1°C for 14–18 hours

2. Homogenize the sample.
 - For soft samples—homogenize thoroughly at 230 rpm for 30 seconds to 1 minute using a homogenizer.
 - For samples containing hard particles, such as bone—squeeze the bag by hand until the sample is mixed thoroughly with the media.
3. Incubate at 41.5±1°C for 14–18 hours.
4. Remove the enriched sample from the incubator, transfer approximately 1.5 mL to a new microcentrifuge tube, then close the tube and briefly mix.

Proceed directly to Chapter 4, “Prepare the lysate”, or store the retained sample at 2–8°C for a maximum of 72 hours. Do not exceed 72 hours of total storage time.

Enrich poultry carcass rinses

1. Pre-warm the media to $41.5 \pm 1^\circ\text{C}$.
2. Transfer the sample to a homogenizer bag, then add the media, as indicated.

Table 11 Enrichment conditions for poultry carcass rinses

Sample size	PCR Instrument	Volume of media/sample	Media	Incubation
30 mL	<ul style="list-style-type: none"> QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v2.0 or later 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later 	90 mL	Pre-warmed Buffered Peptone Water with 12 mg/L novobiocin ^[1]	$41.5 \pm 1^\circ\text{C}$ for 16–20 hours

^[1] 12 mg/L concentration is based on a total volume of 120 mL.

3. Homogenize the sample by hand mixing.
4. Incubate at $41.5 \pm 1^\circ\text{C}$ for 16–20 hours.
5. Remove the enriched sample from the incubator, transfer approximately 1.5 mL to a new microcentrifuge tube, then close the tube and briefly mix.
Retain sufficient sample for confirmation or repeat testing.

Proceed directly to Chapter 4, “Prepare the lysate”, or store the retained sample at $2\text{--}8^\circ\text{C}$ for a maximum of 72 hours. Do not exceed 72 hours of total storage time.

Enrich shell eggs

1. Transfer the sample to a homogenizer bag, then add the media, as indicated.

Table 12 Enrichment conditions for shell eggs

Sample size	PCR Instrument	Volume of media/sample	Media	Incubation
20 eggs	<ul style="list-style-type: none"> QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v2.0 or later 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later 	2 L	Buffered Peptone Water	$37.0 \pm 1^\circ\text{C}$ for 16–20 hours

2. Homogenize the sample by hand mixing.
3. Incubate at $37.0 \pm 1^\circ\text{C}$ for 16–20 hours.
4. Remove the enriched sample from the incubator, transfer 1 mL of enrichment into 10 mL BPW (ISO) and incubate at 37.0°C for an additional 4 hours.
5. Remove the enriched sample from the incubator, transfer approximately 1.5 mL to a new microcentrifuge tube, then close the tube and briefly mix.
Retain sufficient sample for confirmation or repeat testing.

Proceed directly to Chapter 4, “Prepare the lysate”, or store the retained sample at $2\text{--}8^\circ\text{C}$ for a maximum of 72 hours. Do not exceed 72 hours of total storage time.

Enrich environmental samples

Obtain production environment samples

1. Pre-moisten a sterile sampling swab, wipe, or stainless steel sponge.
 - For sampling of areas that have been cleaned or treated with disinfectants and other cleaning agents, use a neutralizing broth, such as Dey-Engley Broth.
 - For other areas, use sterile Peptone Water or other equivalent diluent.
2. Rub the swab, wipe, or stainless steel sponge in both a horizontal and vertical direction across the entire sampling area.
3. Place the sample in the original packaging or other material that is suitable for transport.

Enrich production environment samples

1. Add samples to media, as indicated.

Table 13 Enrichment conditions for production environment samples

Sample type	PCR Instrument	Volume of media/sample	Media	Incubation
Swabs	<ul style="list-style-type: none"> • QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v2.0 or later • 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later 	10 mL	Buffered Peptone Water with 12 mg/L novobiocin	$41.5 \pm 1^\circ\text{C}$ for 14–18 hours
Wipes		100 mL		
Stainless steel sponges		225 mL		
<ul style="list-style-type: none"> • Solid samples • Liquid samples 		225 mL per 25-g or 25-mL sample		

2. Homogenize thoroughly by hand or at 230 rpm using a laboratory blender.

3. Incubate at $41.5 \pm 1^\circ\text{C}$ for 14–18 hours.
4. Remove the enriched sample from the incubator, then transfer approximately 1.5 mL to a new microcentrifuge tube, then close the tube and briefly mix.
Retain sufficient sample for confirmation or repeat testing.

Proceed directly to Chapter 4, “Prepare the lysate”, or store the retained sample at $2\text{--}8^\circ\text{C}$ for a maximum of 72 hours. Do not exceed 72 hours of total storage time.

4

Prepare the lysate

1. Equilibrate the Lysis Reagent 1 Tubes to room temperature.
 - a. Place the required number of Lysis Reagent 1 Tubes in a MicroAmp™ Splash-Free Base and MicroAmp™ Tray.
 - b. Check that there is no liquid around the plastic seal and the reagents are collected at the bottom of each tube.
 - c. Allow the tubes to remain at room temperature for approximately 10 minutes before opening.

2. Remove the plastic seal from each Lysis Reagent 1 Tube, then add 10 µL of Proteinase K to the tube.

These tubes are referred to as Lysis Tubes in the rest of the procedure.

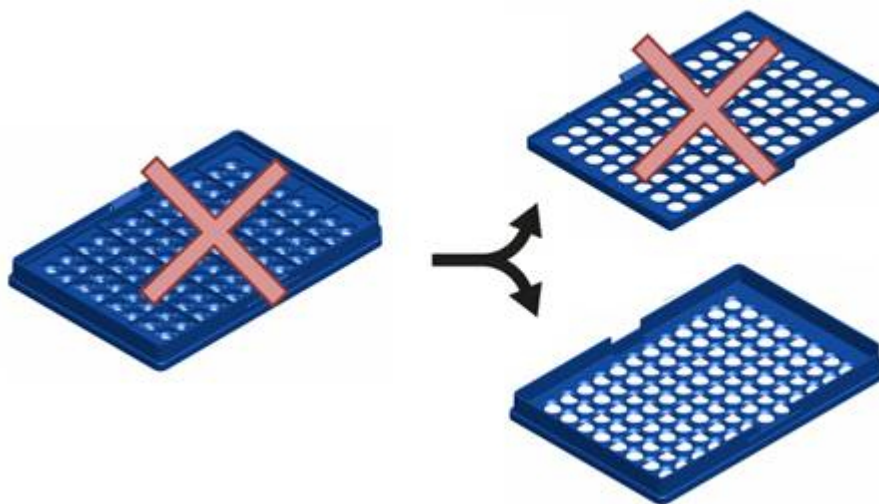
IMPORTANT! Avoid contamination of the Proteinase K stock tube. Use a new filtered pipette tip each time Proteinase K is withdrawn from the stock tube. Use a 10–100 µL repeat pipettor to reduce the number of tips required.

3. Transfer 10 µL of the enriched sample to a Lysis Tube. For the negative extraction controls, transfer 10 µL of sterile enrichment media to a Lysis Tube.

Ensure that the pipette tip reaches the bottom of the Lysis Tube, to facilitate complete mixing of the sample with the lysis reagents.

4. Seal the tubes with the domed Lysis Tube Caps using the capping tool, then incubate the samples in the SimpliAmp™ Thermal Cycler using the following program.

IMPORTANT! To prevent crushing the tubes in the SimpliAmp™ Thermal Cycler, use the bottom piece from the MicroAmp™ 96-Well Tray/Retainer Set or include at least 4 complete SureTect™ Lysis tube strips (see “Guidelines for sample lysis” on page 15).



Ensure that the lid heater is on and set to 105°C, and the volume is set to Maximum.

Step	Temperature	Time
1	37°C	10 minutes
2	95°C	5 minutes
3	10°C	2 minutes
4	4°C	Hold ^[1]

^[1] For convenience, samples can be held at 4°C until proceeding to PCR or transfer to storage at 2–8°C.

5. Proceed directly to Chapter 6, “Perform PCR amplification”.

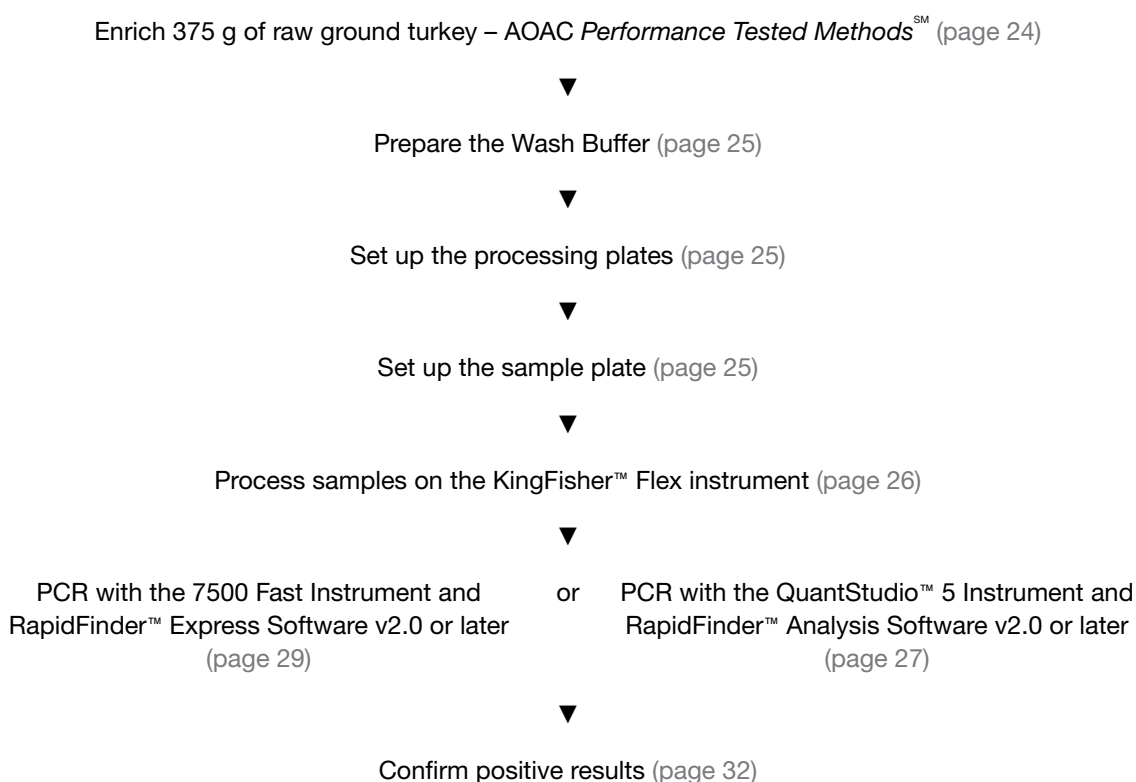
(Optional) Store the samples at 2–8°C for up to 24 hours, including any time stored at 4°C in the thermal cycler.



Enrich and isolate DNA from 375-g ground turkey samples

Use the RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex Flex Kit (Cat. No. A33227KF) for this procedure.

Workflow



Procedural guidelines

Guidelines for sample enrichment

- For preparation of master suspensions, follow the instructions of EN ISO 6887 and EN ISO 6579 standards. Comply with Good Laboratory Practices (refer to EN ISO 7218 standard).
- Follow the manufacturer's instructions for preparation of culture media.

- For consistent PCR results:
 - Use a ventilated incubator.
 - Follow the specified temperature and time allowances.
- Dispose of all inoculated culture media as hazardous microbiological waste, even if shown to be negative for the target organism, according to local guidelines.

Guidelines for sample lysis

- Maintain a homogeneous suspension of the magnetic beads, because this maximizes the surface area to which the target organism can bind. The appearance of the mixture should be homogeneous after mixing.
- Prepare a mock-purified sample, using sterile media (such as BPW) instead of enriched sample, as a negative extraction control. (The negative extraction control is required for RapidFinder™ Express Software; it is optional but recommended for RapidFinder™ Analysis Software.)

Enrich 375 g of raw ground turkey – AOAC Performance Tested MethodsSM

1. Pre-warm the media to 41.5±1°C.
2. Transfer the sample to a homogenizer bag, then add the media, as indicated.

Sample size	PCR Instrument	Volume of media/sample	Media	Incubation
375 g	<ul style="list-style-type: none"> • QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v2.0 or later • 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later 	1.125 L	Pre-warmed Buffered Peptone Water with 12 mg/L novobiocin	41.5±1°C for 20–24 hours

3. Homogenize the sample thoroughly at 230 rpm for 30 seconds to 1 minute using a homogenizer.
4. Incubate at 41.5±1°C for 20–24 hours.
5. Remove the enriched sample from the incubator, transfer approximately 1.5 mL to a new microcentrifuge tube, then close the tube.
Proceed directly to “Set up the processing plates” on page 25, or store the retained sample at 2–8°C for a maximum of 72 hours. Do not exceed 72 hours of total storage time.

Prepare the Wash Buffer

1. Combine the following components, then mix well by inversion or vortexing.

Component	Amount ^[1]
Oxoid™ Phosphate Buffered Saline Tablet	1 tablet
Deionized water	100 mL
Tween™ 20	50 µL

^[1] Sufficient for up to 40 samples. Triple the amounts for up to 96 samples.

2. Sterilize by autoclaving at 115°C for 10 minutes.

Store the prepared Wash Buffer at room temperature (20–25°C).

Set up the processing plates

Set up the processing plates as described in the following table:

Plate	Plate type	Action
Tip Comb	Deep Well	Place a 96-well Deep Well Tip Comb in the plate.
Elution Plate	Deep Well	Add 180 µL Lysis Reagent 1 and 10 µL Proteinase K to each sample and control well. ^[1,2]
Wash Plate 1	Deep Well	Add 1000 µL of Wash Buffer to each sample and control well.
Wash Plate 2	Deep Well	Add 1000 µL of Wash Buffer to each sample and control well.

^[1] Ensure Lysis Reagent 1 is thoroughly mixed while dispensing to ensure even distribution of particles in the reagent.

^[2] (Optional) Combine Lysis Reagent 1 and Proteinase K for the required number of reactions, plus overage, then add 190 µL to each sample and control well.

Set up the sample plate

Use a deep-well plate.

1. Add 500 µL of Wash Buffer to each sample and control well.
2. Add 25 µL Dynabeads™ anti-Salmonella to each sample and control well.
3. Add 500 µL of enriched sample to the appropriate sample well.
4. Add 500 µL of sterile media (such as BPW) to one or more wells to prepare a mock-purified (negative extraction control) sample.

Process samples on the KingFisher™ Flex instrument

1. Select the **A33227KF_RF_Sal** program on the instrument, then press **Start**.
2. Load the prepared plates according to the readout on the instrument, verifying that their orientation is (A1 to A1).
3. When the run is complete, remove the Elution Plate from the instrument.
The Elution Plate contains the sample lysate.

Note: To remove possible viable pathogens before disposal, autoclave the sample and wash the plates.

4. Proceed directly to PCR.
 - See “PCR with the 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later” on page 29.
 - See “PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v2.0 or later” on page 27.

After removal of sample lysate for PCR, seal the Elution Plate with a plate seal. *(Optional)* store the plates at 2–8°C for up to 24 hours.



Perform PCR amplification

PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v2.0 or later

Set up the plate layout in RapidFinder™ Analysis Software

The plate layout is determined by the user. See the Help function in the software for detailed instructions.

In the home screen of RapidFinder™ Analysis Software, click **Create Experiment**, then enter or edit the well parameters.

Select **RapidFinder Salmonella SP-SE-ST Multiplex version 2.0** or later for the assay.

Set up the PCR reactions

Before starting this procedure, ensure that you are familiar with “Guidelines for PCR” on page 15.

1. Following the plate layout previously set up in the software, place the required number of Salmonella Multiplex (Sp/SE/ST) PCR Tubes (PCR tubes) in the MicroAmp™ 96-Well Tray for VeriFlex™ Block. Place the block on the MicroAmp™ Splash-Free 96-Well Base. Press the PCR tubes to the tray to ensure they sit firmly, then tap the tubes on the bench to ensure that the pellets are located at the bottom of the tubes.
2. Allow the tubes to remain on the bench for approximately 5 minutes, to bring to room temperature (23±5°C), then open one strip of PCR tubes by removing the seal.

IMPORTANT!

- If all samples lysates can be applied to the PCR tubes in 10 minutes, then open *all* strips of the PCR tubes.
 - If all samples lysates cannot be applied to the PCR tubes in 10 minutes, then open *only one* strip, the proceed to the next step.
-

PCR pellets are pale yellow. Do not use the pellet if it is collapsed or not pale yellow.

If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty, pipette tip. Do not use a tip containing lysate.

3. Open the Lysis Tubes using the decapping tool. If using an Elution plate, uncover the plate.

- Transfer 20 µL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet.

IMPORTANT! Remove lysate from the top half of the liquid to ensure that no lysis particles are transferred from the Lysis Tube to the PCR tube. Do not touch the pellet when adding the lysate.

- Seal the PCR tubes with the flat optical PCR Caps provided with the kit.
Ensure that the tubes are properly sealed by pressing down firmly over each opening.
- If *only one* strip of PCR tubes was opened, then repeat steps 2–5 for the remaining strips of PCR tubes.
- Mix all PCR tubes thoroughly for 10–15 seconds to ensure that the pellet is fully rehydrated.
Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. If needed, hold the tubes upright, and flick sharply downward.

IMPORTANT! Start the PCR run within 30 minutes of addition of sample lysates to the PCR tubes.

Load and run the reactions

- Eject the instrument drawer. Use the MicroAmp™ 96-Well Tray for VeriFlex™ Block to transfer the tubes to the instrument in the same configuration as the plate layout determined in the software, then close the instrument drawer.
- In the **Run** tab of the experiment file in RapidFinder™ Analysis Software, select the instrument's serial number from the **Instrument** drop-down list.
- Click **Start Run**, then follow the software prompts.




View results and data analysis

Data analysis is automated by the software. For detailed instructions, and options for reporting, export, and storage of results, see the Help function in the software.

In the home screen of the RapidFinder™ Analysis Software, click **Results**, then click the sub-tab for the desired view of the data.

- Summary**—plate format
- Results**—table format
- Details**—amplification plot

RapidFinder™ Analysis Software results icons

Result icon	Result
	Positive result
	Negative result
	Result warning

PCR with the 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later

Set up the plate layout in RapidFinder™ Express Software

RapidFinder™ Express Software determines the Run Layout (plate layout) for your samples based on the information entered, and creates a run file. Refer to the Help function in the software for more details.

On the main page of RapidFinder™ Express Software, select **Create/Edit a Run File**, then enter or edit the Run File information at the prompts.

If desired, you can manually customize the plate layout in the software.

Select **ST_Multiplex_RapidFinder_7500_2.0 or later** for the target.

Set up the PCR reactions

Before starting this procedure, ensure that you are familiar with “Guidelines for PCR” on page 15.

1. Following the plate layout previously set up in the software, place the required number of Salmonella Multiplex (Sp/SE/ST) PCR Tubes (PCR tubes) in a suitable rack with a PCR carry plate, then tap the rack of tubes on the bench to ensure that the pellets are located at the bottom of the tubes.

If required by the plate layout, place empty low profile PCR tubes in the rack; these balance the tray when the tubes are placed in the instrument.

2. Allow the tubes to remain on the bench for approximately 5 minutes, to bring to room temperature ($23\pm5^{\circ}\text{C}$), then open one strip of PCR tubes by removing the seal.

IMPORTANT!

- If all samples lysates can be applied to the PCR tubes in 10 minutes, then open *all* strips of the PCR tubes.
 - If all samples lysates cannot be applied to the PCR tubes in 10 minutes, then open *only one* strip, the proceed to the next step.
-

PCR pellets are pale yellow. Do not use the pellet if it is collapsed or not pale yellow.

If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty, pipette tip. Do not use a tip containing lysate.

3. Open the Lysis Tubes using the decapping tool. If using an Elution plate, uncover the plate.
4. Transfer 20 μL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet.

IMPORTANT! Remove lysate from the top half of the liquid to ensure that no lysis particles are transferred from the Lysis Tube to the PCR tube. Do not touch the pellet when adding the lysate.

5. Seal the PCR tubes with the flat optical PCR Caps provided with the kit.
Ensure that the tubes are properly sealed by pressing down firmly over each opening.

6. If *only one* strip of PCR tubes was opened, then repeat steps 2–5 for the remaining strips of PCR tubes.
7. Mix all PCR tubes thoroughly for 10–15 seconds to ensure that the pellet is fully rehydrated. Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. If needed, hold the tubes upright, and flick sharply downward.

IMPORTANT! Start the PCR run within 30 minutes of addition of sample lysates to the PCR tubes.

Load and run the reactions

In the RapidFinder™ Express Software, select **Start Instrument Run**  on the main page, select the appropriate run file, and follow the software prompts.

1. Use the PCR carry plate to transfer the tubes to the instrument in the same configuration as the run layout.
Use the 7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips in the instrument.
Be sure to load empty low profile PCR tubes as directed by the software (Figure 2).
2. Close the tray to the instrument, and follow the RapidFinder™ Express Software prompts to start the run.

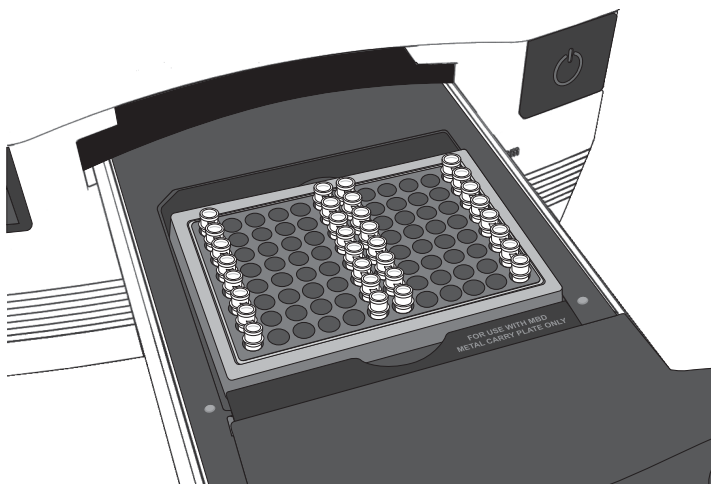



Figure 2 7500 Fast instrument tube layout

RapidFinder™ Express Software directs the user to load empty strip tubes in column 1 (far left) and column 12 (far right), if needed. 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.




View results and data analysis

Data analysis is automated by the software.

In the RapidFinder™ Express Software, select **View Results**  on the main page, select the appropriate run file, and follow the prompts to view results.

To display a list of results in table format, click **Table View**. Select a sample, then click **View Details** to see replicate information about samples.

RapidFinder™ Express Software results icons

Result icon ^[1]	Result
	Positive result
	Negative result
	Result warning

^[1] RapidFinder™ Express displays results pictorially.

Options for reporting results

See the RapidFinder™ Express Software Help function for options for reporting, export, and storage of results.

Recommended confirmation methods

In the context of AOAC *Performance Tested Methods*SM certification, samples with presumptive PCR positive results should be tested further using one of the following methods:

- Serological testing (see “Serological confirmation” on page 32).
- The appropriate reference method for the sample matrix (see “AOAC *Performance Tested Methods*SM Certification” on page 39).

In the event of a positive PCR result, which cannot be confirmed using the steps described above, all necessary measures must be taken by the laboratory to establish the true status of the sample before reporting the result.

Serological confirmation

Overview

Salmonella serotypes Typhimurium Group O:4 (B) and Enteritidis Group O:9 (D1) are identified using antisera to O and H antigens (Table 14).

Table 14 Antigen testing for *Salmonella* serotypes Typhimurium and Enteritidis

Antigen	Positive PCR result	
	S. Typhimurium (Group B)	S. Enteritidis (Group D1)
Somatic (O)	O:4	O:9
Flagellar (H)	H (i)	H (g,m)
	H (1,2)	H (1,7)

Many *Salmonella* serotypes possess diphasic H antigens, but usually only one H antigen is expressed. To detect the other H antigen, expression of the alternate phase is required. Both phases of identifying H antigens must be tested to confirm the identity of any *Salmonella* isolate.

In this procedure, a presumptive positive isolate in liquid suspension is subcultured in the presence of alternate antisera (Table 15), to induce expression of the alternate phase antigen. For example, for *Salmonella* Typhimurium, subculture in the suspension with H (i) antiserum induces expression of H (1,2) antigens. Expression of H (1,2) antigens is checked using H (1,2) antiserum.

Table 15 Alternate phase testing of H antigens

Somatic antigen result	Subculture in the presence of this antiserum	Check for expression of antigen(s) with this antiserum
O:4 positive (<i>S. Typhimurium</i>)	H (i)	H (1,2)
	H (1,2)	H (i)
O:9 positive (<i>S. Enteritidis</i>)	H (g,m)	H (1,7)
	H (1,7)	H (g,m)

Isolate presumptive positive *Salmonella* colonies

1. Streak 10 µL from the stored enriched sample onto *Brilliance*™ *Salmonella* Agar and incubate for 22–26 hours at 37±1°C.
2. (Optional) If there is high background on the plate, perform additional selective plating:
 - a. Subculture 0.1 mL of the stored enriched culture into 10 mL of Rappaport-Vassiliadis Soya (RVS) Broth and incubate at 41.5±1°C for 21–27 hours.
 - b. Plate out 10 µL of the incubated RVS Broth onto *Brilliance*™ *Salmonella* Agar and incubate at 37±1°C for 22–26 hours.
3. Confirm well-isolated, presumptive positive *Salmonella* species colonies using the Oxoid™ *Salmonella* Test Kit (latex test).
Some strains of *Salmonella* ser. Dublin may show weak pigmentation on *Brilliance*™ *Salmonella* Agar, because of their low esterase activity.
For samples with presumptive *S. Typhimurium* or *S. Enteritidis* PCR results, continue with antigen testing.

For samples with a positive PCR test for *Salmonella* species only, and a positive latex test, the test sample is confirmed as positive for *Salmonella* species. No further antigen testing is required.

Perform antigen testing

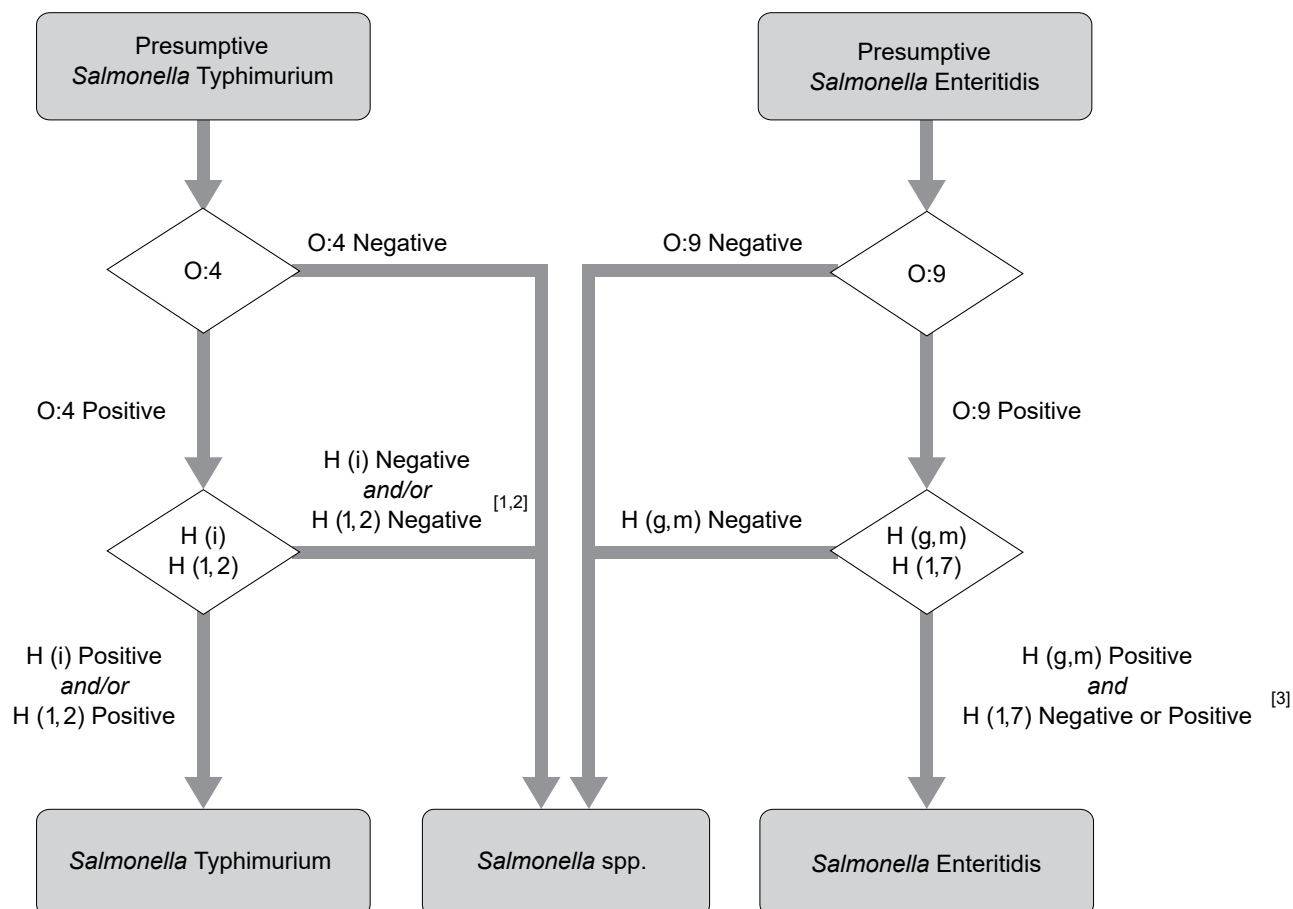
1. Emulsify a well-isolated colony from the *Brilliance*™ *Salmonella* Agar plate in 1 mL of saline.
2. Transfer 400 µL of the liquid suspension to the base of each of 2 Nutrient Agar slopes.
3. Streak from the liquid suspension up the slope of the agar.
4. Pipet 10 µL of the required antiserum into the liquid suspension at the base of each Nutrient Agar slope.
5. Incubate the slopes for 18–24 hours at 37±1°C.

6. Sample growth from the top half of the Nutrient Agar slope, then test for the expected somatic O antigen (Table 14).

If the sample is negative for the O antigen, the organism is identified as *Salmonella* spp. Do not proceed to flagella H antigen testing (next step).

7. If the sample is positive for the O antigen, sample the liquid suspension at the base of the Nutrient Agar slope using a 10-μL loop, then test for identifying H antigens (Table 15).

See Figure 3 for interpretation of results.



[1] Rare cases of *Salmonella* Typhimurium may be monophasic, that is, may or may not possess H (i) or H (1,2) antigens.

[2] Exceptional cases of *Salmonella* Typhimurium may be non-motile, that is, may not possess any H antigens.

[3] Most strains of *Salmonella* Enteritidis are negative for the H (1,7) antigen. Exceptional cases may express H (1,7) antigen.

Figure 3 Somatic and flagella antigen testing: interpretation of results

Confirmation of the exceptional cases described in the figure may be conducted by a reference laboratory.



Troubleshooting

Observation	Possible cause	Recommended action
Dynabeads™ anti-Salmonella IMS beads form a white precipitate in the tube	There are particles in the tube that are not yet dissolved.	Incubate the tube containing the IMS particles at 37±1°C for approximately 10 minutes.
		If the white precipitate is not completely dissolved after 10 minutes at 37°C, apply longer incubation times and higher temperatures (up to 50°C).
In negative extraction control wells, target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred.	<ol style="list-style-type: none"> 1. Repeat the assay using fresh aliquots of all reagents, fresh enrichment, and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
In negative extraction control wells, no IPC signal is detected, but a target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred. Additionally, a problem with the IPC occurred due to: <ul style="list-style-type: none"> • Preferential amplification of the carryover DNA. • Carryover of particles from the Lysis Tube. 	<ol style="list-style-type: none"> 1. Repeat the assay using fresh aliquots of all reagents, fresh enrichment, and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
In negative extraction control wells, no IPC signal or an exceptionally weak or atypical IPC amplification plot is detected. The result is considered invalid by the software.	Pellets were not fully dissolved and/or lysate was not at the bottom of the tube before the PCR run was started.	Mix thoroughly by vortexing for 10–15 seconds to ensure the pellet is fully rehydrated and/or lysate is at the bottom of the tube.
	Incomplete lysis steps caused an inhibition of the PCR.	Retest the original sample and diluted sample, ensuring that the correct heating parameters are followed.

Observation	Possible cause	Recommended action
In test samples, no IPC nor target-specific signal is detected, and/or an exceptionally weak or atypical amplification plot is observed. The result is considered invalid by the software.	Inhibition of PCR occurred, due to: <ul style="list-style-type: none"> • Carryover of particles from the Lysis Tube. • PCR inhibitors present in the food sample. • Incomplete sample lysis. • Other, unknown, cause. 	Retest the original sample and its dilution. To remove the impact of PCR inhibitors in the sample, dilute the enriched sample 1:5 (1 part enriched sample and 4 parts sterile media), or 1:10 (1 part enriched sample and 9 parts sterile media), then repeat the sample lysis procedure and PCR.
	Pellets were not fully dissolved and/or lysate was not at the bottom of the tube before the PCR run was started.	Mix thoroughly by vortexing for 10–15 seconds to ensure the pellet is fully rehydrated and/or lysate is at the bottom of the tube.
	Bubbles were present in the PCR tube.	Inspect each tube for bubbles by looking through the optical PCR Caps. Large bubbles can often be removed by firmly holding the top of the tube while gently flicking the bottom. If the bubble persists, spin the tube for 10 seconds in a plate spinner. If the bubble continues to persist, set up a new PCR tube using the prepared lysate.
In test samples, no IPC signal is detected, but target-specific signal is detected. The result is considered invalid by the software.	A problem occurred in IPC amplification due to preferential amplification of the target-specific DNA.	Retest the original sample and diluted sample. Dilute the enriched sample 1:5 (1 part enriched sample and 4 parts sterile media) or 1:10 (1 part enriched sample and 9 parts sterile media), then repeat the sample lysis procedure and PCR.
In test samples that are expected to be positive, no target-specific signal is detected.	Certain sample types contained components that were inhibitory to the growth of the target organism.	Dilute the sample pre-enrichment to a level that prevents growth inhibition of target bacteria. For example, following guidelines in ISO 6887.
In test samples, signal is detected for <i>S. Typhimurium</i> or <i>S. Enteritidis</i> , but no signal is detected for <i>S. spp.</i> The software returns a result warning.	The copy number of the <i>S. Typhimurium</i> or <i>S. Enteritidis</i> target is not high enough to give a strong positive signal for <i>S. spp.</i>	Incubate the retained sample of enriched culture for an additional 2–4 hours (to increase the level of <i>S. Typhimurium</i> or <i>S. Enteritidis</i> in the culture), then repeat the lysis and PCR.
		If signal is detected during repeat testing for <i>S. Typhimurium</i> or <i>S. Enteritidis</i> , but no signal is detected for <i>S. spp.</i> , confirm the presence of <i>S. Typhimurium</i> or <i>S. Enteritidis</i> using the recommended culture confirmation protocol. Confirm the presence of <i>S. Typhimurium</i> or <i>S. Enteritidis</i> using the recommended culture confirmation protocol.

Observation	Possible cause	Recommended action
In test samples, serological confirmation of <i>S. Enteritidis</i> or <i>S. Typhimurium</i> does not match PCR result	The sample is contaminated with more than one <i>Salmonella</i> serotype.	Return to the <i>Brilliance</i> ™ <i>Salmonella</i> Agar plate and inspect for multiple colony morphologies. Repeat the confirmation procedure with each colony morphology.
		<ol style="list-style-type: none"> 1. Return to the <i>Brilliance</i>™ <i>Salmonella</i> Agar plate and, using a sterile loop, pick growth from the primary bed, then suspend in 1 mL of sterile saline. 2. Process the suspended sample through lysis and PCR. 3. If the positive result is maintained, subculture the suspension onto <i>Brilliance</i>™ <i>Salmonella</i> Agar and repeat the serological confirmation.
		<ol style="list-style-type: none"> 1. Return to the retained enrichment sample and perform a serial dilution (for example, to 10⁻⁴) using a suitable diluent (for example, BPW/MRD). 2. Plate each dilution onto <i>Brilliance</i>™ <i>Salmonella</i> Agar using a spread plate technique. Inspect for morphologically distinct, suspect colonies. 3. Repeat the serological confirmation procedure on suspect colonies.
In confirmation testing, suspect colonies on <i>Brilliance</i> ™ <i>Salmonella</i> Agar are not present	Overgrowth of <i>Salmonella</i> with background flora.	Sub-culture 100 µL of the retained enrichment into 10 mL of Rappaport-Vassiliadis Soya (RVS) Broth. Incubate at 41.5 °C for 21–27 hours before continuing with confirmation.
In confirmation testing, suspect colonies on <i>Brilliance</i> ™ <i>Salmonella</i> Agar are too small to conduct a latex test and serological confirmation	The isolate is sensitive to selective components in the medium or the lower limit of the incubation time was used.	Purify the well-isolated, suspect colony on a non-selective plating medium to increase biomass before continuing with confirmation.
In confirmation testing, suspect colonies on <i>Brilliance</i> ™ <i>Salmonella</i> Agar are not well isolated	The enriched sample contains high levels of background flora that were not inhibited on <i>Brilliance</i> ™ <i>Salmonella</i> Agar.	Purify the suspect colonies on a second <i>Brilliance</i> ™ <i>Salmonella</i> Agar plate before continuing with confirmation.

Test control organisms

Incorporation of positive control organisms is not necessary with routine testing of samples, because the PCR results are validated if the IPC signal is detected.

If testing of positive control organisms is required, select suitable organisms. Quality control organisms are available from Thermo Fisher Scientific, Microbiology Division. Contact your local supplier for further information.

- Process control organism in parallel with test samples through sample enrichment, lysis, and PCR.
- Alternatively, omit the enrichment step for the control organisms: take a single colony into 1 mL of sterile saline to form a homogenous suspension. Process the prepared suspension through lysis and PCR with test samples.

RapidFinder™ Express Software results warnings

RapidFinder™ Express Software v2.0 may indicate a result warning due to inhibition for some samples. In some rare cases the warning label is result of **Non-linear baseline** notification for the bacterial targets and/or IPC detector of the assay.

In such rare cases, follow the recommended workflow:

1. Select **View details** to manually view results of the highlighted reaction for the bacterial targets and the IPC in the RapidFinder™ Express Software v2.0.
2. Inspect the IPC result.
3. Inspect the bacterial targets results.

If the C_t of the IPC is below the cut off C_t value depicted in following table and the bacterial targets have received a negative interpretation and the signal is above the cut off C_t value, the result can be interpreted as true negative.

Whenever the IPC and bacterial targets have received C_t values below the cut off C_t values depicted in the following table, proceed to a confirmation step as described in the user guide.

In case of a negative IPC result or IPC C_t above the cut off, follow the instructions given in the user guide to repeat the sample.

Assay	Cut off for target C_t value	Cut off for IPC C_t value
RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit	40 (all targets)	35.5




Supplemental information

AOAC Performance Tested MethodsSM Certification

Go to thermofisher.com/foodsafety for a guide to workflows for detection of *Salmonella* spp. (Pub. No. MAN0009417).

Table 16 Performance Tested MethodsSM Certification of the workflow

Certification
 <p>PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 081701</p>

The detection of *Salmonella* spp., *S. Typhimurium*, and *S. Enteritidis* using RapidFinder™ *Salmonella* species, Typhimurium and Enteritidis Multiplex PCR Kit has earned the AOAC Performance Tested MethodsSM Certification from the AOAC Research Institute. The certified workflow described in this user guide includes:

- Enrichment in Buffered Peptone Water (ISO)
- RapidFinder™ *Salmonella* species, Typhimurium and Enteritidis Multiplex PCR Kit
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 17 for characteristics) with RapidFinder™ Analysis Software v2.0 or later and Pathogen Assay File: **RapidFinder *Salmonella* SP-SE-ST Multiplex version 2.0** or later

Table 17 QuantStudio™ 5 Real-Time PCR Instrument characteristics

Characteristics	QuantStudio™ 5 Real-Time PCR Instrument
Optics	Bright white LED
Filters	6 excitation and 6 emission filters
Sample ramp rate	Average: 3.66°C/sec Maximum: 9.0°C/sec
Thermal range	4-99°C
Thermal accuracy	±0.25°C
Thermal uniformity	±0.4°C
Format	96-well, 0.1-mL block

- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 18 for characteristics) with RapidFinder™ Express Software v2.0 or later, and Pathogen Assay File: **ST_Multiplex_RapidFinder_7500_2.0** or later

Table 18 7500 Fast Real-Time PCR Instrument characteristics

Characteristics	7500 Fast Real-Time PCR Instrument
Optics	12v 75w halogen bulb
Filters	5 excitation and 5 emission filters
Sample ramp rate	Standard mode: $\pm 1.6^{\circ}\text{C}/\text{sec}$ Fast mode: $\pm 3.5^{\circ}\text{C}/\text{sec}$
Thermal range	4-100°C
Thermal accuracy	$\pm 0.5^{\circ}\text{C}$
Thermal uniformity	$\pm 1^{\circ}\text{C}$
Format	96-well, 0.1-mL block

- Confirmation testing of positive samples, as described in “Recommended confirmation methods” on page 32.

Table 19 Validated matrices

Reference method	Matrices
USDA MLG 4.09	<ul style="list-style-type: none"> 25 g raw pork sausage 25 g breaded chicken nuggets 25 g raw chicken wings with skin 25 g raw chicken thighs with skin 375 g raw ground turkey Chicken carcass rinses
US FDA BAM Ch. 5	<ul style="list-style-type: none"> Shell eggs Stainless steel surface sponges

Good laboratory practices for PCR








To avoid amplicon contamination of samples, follow these guidelines when preparing or handling samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified 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 and reaction setup.
 - Amplification and analysis of products.

- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Do not open reaction tubes after PCR.
- Do not autoclave reaction tubes after PCR.
- Clean lab benches and equipment periodically with 10% bleach solution or DNAZap™ Solutions (Cat. No. AM9890).

For additional information, refer to EN ISO 22174:2005 or www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html.

Symbol definitions

Symbol	Definition
	BATCH CODE
	CATALOG NUMBER
	CONTAINS SUFFICIENT FOR <n> TESTS
	CONSULT INSTRUCTIONS FOR USE
	MANUFACTURER
	UPPER AND LOWER TEMPERATURE LIMIT (storage temperature)
	USE BY



Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit [thermofisher.com/support](https://www.thermofisher.com/support).



Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.



- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. 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.

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311



Documentation and support

Food safety support

Website: <https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html> or [thermofisher.com/foodsafety](https://www.thermofisher.com/foodsafety)

Support email:

- Europe, Middle East, Africa: microbiology.techsupport.uk@thermofisher.com
- North America: microbiology@thermofisher.com

Phone: Visit [thermofisher.com/support](https://www.thermofisher.com/support), select the link for phone support, then select the appropriate country from the dropdown list.

Customer and technical support

Visit [thermofisher.com/support](https://www.thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Related documentation

Document	Publication number
<i>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i>	MAN0010407
<i>SimpliAmp™ Thermal Cycler User Guide</i>	MAN0009889
<i>SimpliAmp™ Thermal Cycler Installation and Operation Quick Reference</i>	A24827

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

References

- EN ISO 6579-1:2017. Microbiology of the food chain – Horizontal method for detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp.
- EN ISO 6887-1:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions.
- EN ISO 6887-2:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 2: Specific rules for the preparation of meat and meat products.
- EN ISO 6887-3:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 3: Specific rules for the preparation of fish and fishery products.
- EN ISO 6887-4:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 4: Specific rules for the preparation of miscellaneous products.
- EN ISO 6887-5:2010. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 5: Specific rules for the preparation of milk and milk products.
- EN ISO 7218:2007. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.
- EN ISO 22174:2005. Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – General requirements and definition.
- USDA Microbiology Laboratory Guidebook (MLG) 4.09: Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges. January 2, 2017. <http://www.fsis.usda.gov/wps/wcm/connect/fsis-content/internet/main/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook>
- USFDA Bacteriological Analytical manual (BAM) Chapter 5: *Salmonella*. August 2016. <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm>

