USER GUIDE

applied biosystems by life technologies™

TaqMan® *Salmonella enterica* Detection Kit

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PERFORMANCE TESTED AOAC RESEARCH INSTITUTE
LICENSE NUMBER 020803

BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

ABI 29/01 – 09/07

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

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

Certified by AFNOR Certification

For testing of Food and Environmental samples only.

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TRADEMARKS

Contents

TaqMan® *Salmonella enterica* Detection Kit

IMPORTANT! Before using this product, read and understand the information in Appendix E, "Safety" on page 29 in this document.

Overview

About this protocol

This protocol provides:

- Background material about the TaqMan® *Salmonella enterica* Detection Kit
- A list of materials and equipment that can be used with the TaqMan® *Salmonella enterica* Detection Kit
- Guidelines for sample enrichment and preparation
- Instructions for preparing PCR using the TaqMan® *Salmonella enterica* Detection Kit on an Applied Biosystems Sequence Detection System (SDS) or Real-Time PCR System
- General troubleshooting guidelines

Product description

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

Description of target microorganisms

The TaqMan® *Salmonella enterica* Detection Kit detects all subspecies of *Salmonella enterica* including *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica only*. *Salmonella enterica* causes disease in humans and other warm-blooded animals and therefore incorporates the most important clinical serovars. Hence, this method permits detection of *Salmonella enterica* (Groups I to IV) and is not suitable to detect *Salmonella bongori* (Group V). The genus *Salmonella*, consisting of the two species *Salmonella enterica* and *Salmonella bongori*, is a member of the *Enterobacteriaceae* family. More than 2,400 *Salmonella* serotypes have been reported, all of which are potentially pathogenic. *Salmonella enterica* with its six subspecies is of clinical relevance for humans. *Salmonella* is a frequently reported cause of foodborne illness, occurring in both food poisoning-triggered epidemics and 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 and chocolate, spices, frozen products, and vegetables such as hot peppers.

Kit sensitivity

TaqMan® Pathogen Detection Kits work on enriched samples, and detect as little as 1 CFU in 25 grams of food. The sensitivity of the assay in real culture samples depends on the quality of the sample preparation method that is used.

Kit specificity

The TaqMan® *Salmonella enterica* Detection Kit has demonstrated 100% inclusivity for more than 100 strains of *Salmonella enterica*. It has also demonstrated 100% exclusivity for 30 other non-*Salmonella* strains. For more information, refer to Appendix A, "Specificity" on page 19.

Intended user

The intended users of the TaqMan® *Salmonella enterica* Detection Kit are investigators who need to detect the presence of *Salmonella enterica* in food. The TaqMan® *Salmonella enterica* Detection Kit is for use in food and environmental testing only. Not for any animal or human therapeutic or diagnostic use.

AOAC Performance Tested Methods℠ Certification

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 020803

The TaqMan® *Salmonella enterica* 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:

- The PrepMan® Ultra Sample Preparation Reagent Kit
- The TaqMan® *Salmonella enterica* Detection Kit
- The Applied Biosystems® 7300/7500 Real-Time PCR Systems
- RapidFinder™ Software

The workflow was certified for use with the following matrices:

Reference method	Matrix
ISO 6579	Food matrices: raw ground beef, chicken wings, cheddar cheese, and dry pet food

ISO 16140 Validation

BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

ABI 29/01 – 09/07
[End of validity: refer to certificate at www.afnor-validation.com]

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification

The TaqMan® *Salmonella enterica* 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 TaqMan® *Salmonella enterica* Detection Kit
- The PrepMan® Ultra Sample Preparation Reagent Kit
- The Applied Biosystems® 7000/7300/7500/7500 Fast/7700/StepOnePlus® Real-Time PCR Systems
- RapidFinder™ Software, Sequence Detection System (SDS) Software, and StepOne® Software

Reference method	Matrix
ISO 6579	All food and feed categories 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 TaqMan® *Salmonella enterica* method must be confirmed, from the BPW enrichment broth, by performing one of the following tests:

- By performing an enrichment step in RVS (0.1 mL BPW in 10 mL of RVS broth): incubate at 41.5 ± 1°C for 24 ± 3 hours 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 selective enrichment in MKTTn broth, as 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 TaqMan® *Salmonella enterica* method. It is necessary that the complete protocol of the 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 recommendations:

- Comply with Good Laboratory Practices (GLP; refer to EN ISO 7218 standard).
- We recommend that ISO 6579 and ISO 6887 standards be followed for the preparation of "master suspensions".
- In the context of NF Validation, samples of more than 25 grams have not been tested.

Expiration date

For more information about the expiration date of the "NF Validation" certification, please refer to the certificate, ABI 29/01 – 09/07, available on the website at **www.afnor-validation.com**.

Operational conditions

Altitude

The Applied Biosystems® 7000/7300/7500/7500 Fast/7700/StepOnePlus® Real-Time PCR Systems are for indoor use only and for altitudes not exceeding 2,000 m (6,500 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°C (59°F) 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.
- **Environmental Master Mix (EMM)** – A common reagent for all pathogen detection assays. The EMM is used to prepare the premix solution. It contains the polymerase enzyme that initiates PCR in the presence of the necessary primers and DNA sample.
- **Internal Positive Control (IPC)** – A control present in all reaction wells (contained in the Target Assay Mix). The IPC should always yield a positive result. If it does not, there may be a problem with amplification.
- **Negative control** – Monitors for contamination (unexpected amplification in the absence of a target) and reagent integrity (IPC signal should be present). One negative control is required for each target assay.
- **Polymerase Chain Reaction (PCR)** – Technology used to amplify, or increase the amount of a DNA sequence.
- **Positive control** – Monitors for the expected amplification of a target. Target signal not detected in a positive control well indicates a pipetting error or a problem with amplification. A positive control is optional for each target assay and is not recommended because it can cause cross-contamination.
- **Premix solution** – A solution you prepare that contains Environmental Master Mix (EMM) and Target Assay Mix (TAM).
- **Primer** – A segment of DNA that is complementary to the target DNA sequence or Internal Positive Control DNA sequence. Primer is needed to initiate amplification.
- **Probe** – A segment of DNA that is complementary to the target DNA sequence or Internal Positive Control DNA sequence. The probe is labeled with a reporter dye. When the probe binds to the target or Internal Positive Control, a reaction detected by the Sequence Detection System (SDS) or Real-Time PCR System indicates the presence of the target or Internal Positive Control.
- **Target** – The pathogen being tested.
- **Target Assay Mix (TAM)** – Target-specific reaction for the pathogen detection assay. TAM is used to prepare the premix solution. TAM contains specific primers and probes for the target and the IPC.
- **Unknown sample** – A DNA sample from a food substance that you are testing for the presence of one or more food pathogens.

Chemistry overview

Reaction components

Reaction components include:

- Target Assay Mix
- Environmental Master Mix
- DNA isolated from enrichment culture of raw materials or food samples, which you supply

Polymerase Chain Reaction (PCR)

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

Fluorescence detection

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

Internal Positive Control (IPC)

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

Materials and equipment

Kit contents

The TaqMan® *Salmonella enterica* Detection Kit (Part no. 4366104) contains reagents for 100 reactions. Components are shown in the table below.

Cap Color	Component name	Volume (µL)
	*Salmonella enterica* Target Assay Mix, 1 tube	300
	Negative Control, 1 tube	1000
	2X Environmental Master Mix, 2 tubes	750

Storage

- On receipt, store the 10X *Salmonella enterica* Target Assay Mix at –20°C or below and protect from light. Excessive exposure to light may affect the fluorescent probes.
- Store the Negative Control and 2X Environmental Master Mix at 5 ± 3°C.
- Minimize freeze-thaw cycles.

Shelf life

See expiration date on the outer box label.

Equipment and materials not included

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

Instruments, equipment, consumables, and reagents

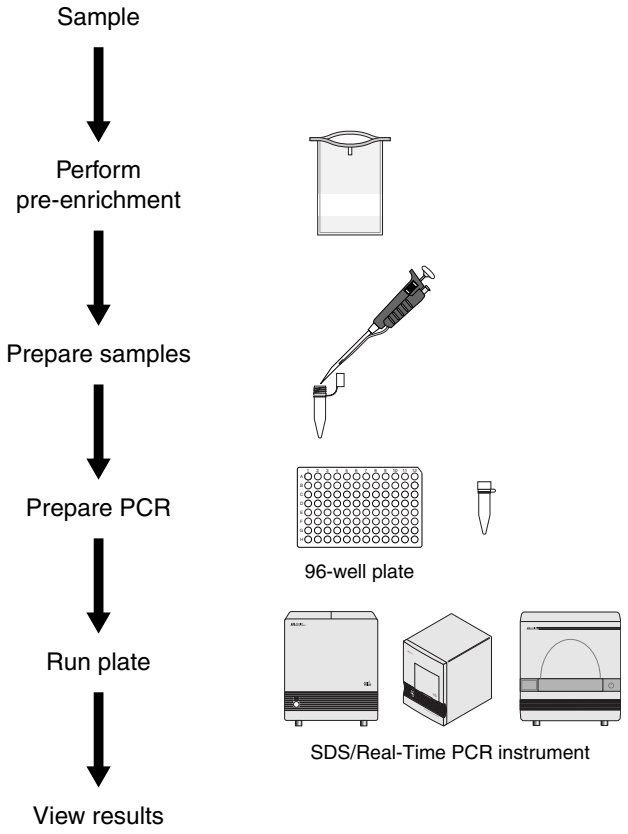
Item	Source
Instruments	
Applied Biosystems® 7000 Real-Time PCR System	Contact your local Life Technologies sales office.
Applied Biosystems® 7300 Real-Time PCR System	
Applied Biosystems® 7500 Real-Time PCR System	
Applied Biosystems® 7500 Fast Real-Time PCR System	
Applied Biosystems® 7700 Real-Time PCR System	
StepOne® Real-Time PCR System	
Equipment	
Benchtop microcentrifuge	MLS
Block heater	MLS
Incubator shaker	MLS
Consumables	
Aerosol-resistant pipette tips	MLS
Disposable gloves	MLS

Instruments, equipment, consumables, and reagents	
Item	**Source**
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
Applied Biosystems® Optical Adhesive Covers, 25 covers	Life Technologies Cat. no. 4360954
MicroAmp® Optical Caps, (8 caps/strip), 300 strips	Life Technologies Cat. no. 4323032
MicroAmp® Optical Adhesive Covers and MicroAmp® 96-Well Optical Reaction Plate with Barcode (code 128), 100 plates with covers	Life Technologies Cat. no. 4314320
MicroAmp® Optical Adhesive Cover Starter Kit	Life Technologies Cat. no. 4313663
MicroAmp® 96-Well Optical Reaction Plate with Barcode (code 128), 20 plates	Life Technologies Cat. no. 4306737
Sterile microcentrifuge tubes with attached screw-cap lid	MLS
Stomacher	MLS
Stomacher bags	MLS
Reagents	
PrepMan® Ultra Sample Preparation Reagent	Life Technologies Cat. no. 4318930
Buffered Peptone Water (BPW)	MLS
RNase-free, sterile-filtered water	MLS
Salmonella enterica subsp. *enterica* serovar *Typhi* (Source strain *Ty2*)‡	ATCC Cat. no. 700931D

‡ Provided for use as a positive control. We do not recommend the use of positive controls with TaqMan® Pathogen Detection Kits. Refer to "Internal Positive Control (IPC)" on page 9 for more information.

Kit workflow

The TaqMan® *Salmonella enterica* Detection Kit workflow is shown below.



Perform pre-enrichment

Overview

Pre-enrichment is the first step in using the TaqMan® *Salmonella enterica* Detection Kit. Pre-enrichment consists of growing the specific pathogen from a selected sample. The recommended enrichment procedure is based on ISO method 6579. For more information, refer to Appendix C, "References" on page 25.

Pre-enrichment process

To perform pre-enrichment:

1. (*Optional*) Prepare an enrichment control, or an incubated pre-enrichment broth without any sample, as a negative control.
2. Warm the Buffered Peptone Water (BPW) broth to 37 ±1°C.
3. Perform pre-enrichment:
   For ground beef, cheddar cheese, and dry pet food:
   a. Combine the sample with BPW broth in a 1:9 ratio; up to 25 grams of sample in 225 mL of BPW broth.

b. Close the stomacher bag, then place in a stomacher for at least one minute and homogenize at normal speed.

c. Incubate the stomacher bag at 37 ±1°C for 18 ±2 hours.

For chicken wings:

a. Add whole chicken wings to a filtered stomacher bag followed by 225 mL of BPW broth.

b. Close the stomacher bag, then hand-massage it for 30 ±15 seconds.

c. Incubate the stomacher bag at 37 ±1°C for 18 ±2 hours.

Note: We recommend the use of filtered stomacher bags to prevent pipetting inaccuracy due to particulants.

Prepare samples

Overview

Sample preparation is the second step in using the TaqMan® *Salmonella enterica* Detection Kit. Sample preparation consists of preparing the pre-enriched sample for inclusion in your PCR.

Sample preparation process

WARNING! CHEMICAL HAZARD. PrepMan® Ultra Sample Preparation Reagent contains a material that can cause eye, skin, and respiratory tract irritation; and adverse effects on the kidneys, blood, and central nervous system. Read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare the pre-enriched sample:

1. Transfer 1 mL of pre-enriched sample from the sample stomacher bag to a sterile microcentrifuge tube, then cap the tube. Use caution when handling enriched samples. Treat samples as biohazard waste.

   Note: Sterile microcentrifuge tubes with attached screw-cap lids are recommended to avoid:
   - Lids opening during sample heating
   - Sample cross-contamination (resulting from the removed lids mistakenly placed on the wrong sample tubes)

2. Centrifuge the tube at maximum speed in a microcentrifuge for 4 ± 1 minutes to spin down the contents.

3. Remove the supernatant from the tube, leaving the pellet behind. Dispose of the supernatant according to biohazardous material disposal procedures.

   Note: Do not disturb the pellet during supernatant extraction.

4. Add 100 µL of PrepMan® Ultra Sample Preparation Reagent, resuspend the pellet, then vortex the tube for 10 ± 5 seconds to mix the contents.

5. Heat the tube in a heat block at 100 ±5°C for 12 ±2 minutes, then vortex the tube for 10 ± 5 seconds to mix the contents.

   Note: Incubate at 95 ±5°C to reduce the frequency of the Eppendorf lids from popping open during this step.

6. Centrifuge the tube at maximum speed in a microcentrifuge for 4 ±1 minute to spin down the contents.
7. Transfer 50 µL of the supernatant into a new microcentrifuge tube. The sample can be stored at 5 ±3°C for up to a month.

   Note: Do not disturb the pellet during supernatant extraction.
8. Dilute the sample DNA with RNase-free water by combining 10 µL of sample DNA with 90 µL of RNase-free water.
9. Vortex the new tube for 10 ±5 seconds to mix the contents. The sample DNA is now ready for PCR.

Prepare PCR

Overview

Preparing PCR is the third step in using the TaqMan® *Salmonella enterica* Detection Kit. Preparing PCR consists of creating the plate document and the reaction mix. The procedure for preparing PCR changes depending on whether or not you use RapidFinder™ Software.

Note: All TaqMan® Pathogen Detection Kits run with a single standard PCR protocol, allowing them to be combined on the same plate. This feature allows screening for multiple pathogens in the same PCR run.

IMPORTANT! If you use RapidFinder™ Software, refer to the "Create or Edit Plate and Pipette a Plate" instructions in the *RapidFinder™ Express Software Online Help*.

⚠ **CAUTION!** CHEMICAL HAZARD. PrepMan® Ultra Sample Preparation Reagent contains a material that can cause eye, skin, and respiratory tract irritation; and adverse effects on the kidneys, blood, and central nervous system. Read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparing PCR (without RapidFinder™ software)

To prepare the reaction mix:

1. Create and set up a plate document with thermal cycling conditions specified in the following table. Refer to the appropriate instrument user guide for more details.

Step	AmpliTaq Gold® enzyme activation	PCR	
	HOLD	Cycle (45 cycles)	
		Denature	Anneal/extend
Temp.	95°C	95°C	60°C
Time	10 min	15 sec	1 min

2. Thaw all reagents completely.
3. Label a tube with the target name.

4. Create the Premix Solution according to the following table.

Component	Vol. (µL) for one 30-µL reaction	Vol. (µL) for four 30-µL reactions‡
2X Environmental Master Mix (EMM)	15.0	66.0
10X Target Assay Mix (TAM)	3.0	13.2
Total Volume	18.0	79.2

‡ This example is illustrative for 4 reactions. Volumes shown include 10% overage to compensate for pipetting errors.

Note: Use a new tip when pipetting EMM and TAM.

Note: At least one negative control is required per target organism tested. Inclusion of a positive control is optional.

5. Mix the solution by gently pipetting up and down, then cap the tube.
6. Repeat steps 3 through 5 for each target assay.
7. Transfer 18 µL of Premix Solution into each well to be used, gently pipetting at the bottom of the well.

   Note: Use a new tip for each Target Premix Solution.

   Note: Refer to "Plate layout suggestions" on page 28 for more information.

IMPORTANT! Steps 8–10: Mix very gently with the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination.
Use a new tip for each well.

8. Transfer 12 µL of unknown sample (from step 9 on page 14) into each sample well, gently pipetting up and down to mix the solution.

IMPORTANT! Steps 8–10: Mix very gently with the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination.

9. Transfer 12 µL of negative control into each negative-control well, gently pipetting up and down to mix the solution.
10. (*Optional*) Close all unknown sample and negative control tubes. Transfer 12 µL of positive control into each positive-control well, gently pipetting up and down to mix the solution.

    Note: Use a new tip for each well, even when pipetting the same positive control.

    Note: We recommend diluting the positive control with RNase-free water to 1 pg/mL, resulting in approximately 2,000 copies.
11. Close the tubes or apply an optical cover to the plate.
12. Make sure that the reagents are in the bottom of the wells. If available, use a centrifuge with a plate adapter to briefly centrifuge the plate.

Run the reactions

Overview

Running a plate is the fourth step in using the TaqMan® *Salmonella enterica* Detection Kit. Running a plate involves using an Applied Biosystems Sequence Detection System (SDS) or Real-Time PCR System to analyze your sample.

IMPORTANT! If you use RapidFinder™ Software, refer to the "Running a Plate" instructions in the *RapidFinder™ Express Software Online Help* (Pub. no. 4401842).

Running a plate without RapidFinder™ Software

To run the plate:

1. Open the plate document that corresponds to the reaction plate.
2. Load the reaction plate into the SDS or Real-Time PCR System.
3. 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

Viewing results varies depending on the instrument you use. Refer to the appropriate instrument user guide of your real-time PCR instrument or the *RapidFinder™ Software Online Help* (Pub. no. 4401842) 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* (Pub. no. 4401842).

General process without RapidFinder™ Express Software

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

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

FAM™ dye signal (target)	VIC® dye signal (IPC)	Result
+	+, –	Positive
–	+	Negative
–	–	See Appendix B, "Troubleshooting" on page 23.

Resources for viewing results

For more information about analyzing your data, refer to:

- The appropriate instrument user guide
- *RapidFinder™ Express Software Online Help* (Pub. no. 4401842)

We do not recommend using the same method to both screen samples and confirm the results. The confirmation methods that can be used (for confirmation of positive results) in the context of NF Validation are described on pages 6 and 7.

A Specificity

Inclusivity

Genus serotypes	Source‡
Salmonella Abaetetuba	Abaetetuba Food Company
Salmonella Abony	NCTC 6017
Salmonella Adelaide	Silliker
Salmonella Agona	Silliker
Salmonella Ajiobo	Ajiobo Food Company
Salmonella Alachua	Silliker
Salmonella Albany	CDC 151-86
Salmonella Anatum	Silliker
Salmonella Anatum	ATCC 9270
Salmonella Arizonae	CDC 346-86
Salmonella Bareilly	Silliker
Salmonella Bere	CDC 315-86
Salmonella Berta	CDC 212-86
Salmonella Betioky	CDC 946
Salmonella Binza	Silliker
Salmonella Bockenheim	CDC 1097
Salmonella Bovismorbificans	CDC 889-83
Salmonella Brandenburg	Brandenburg Food Company
Salmonella Bredeney	Bredeney Feed Company
Salmonella California	ATCC 23201
Salmonella Cerro	ATCC 10723
Salmonella Choleraesuis	ATCC 10708
Salmonella Choleraesuis Kunzendorf	ATCC 12011
Salmonella Crossness	CDC 1795
Salmonella Cubana	Silliker
Salmonella Derby	Derby Feed Company
Salmonella Drypool	Silliker
Salmonella Eastbourne	Silliker
Salmonella Enteritidis	ATCC 4931
Salmonella Ferlac	ATCC 43976
Salmonella Flint	CDC 16-86
Salmonella Gallinarum	CDC 2950-79
Salmonella Gaminara	ATCC 8324
Salmonella Glostrup	ATCC BAA 556
Salmonella Hadar	ATCC 51956
Salmonella Havana	Silliker
Salmonella Heidelberg	ATCC 8326

Genus serotypes	Source‡
Salmonella Hilversum	ATCC 15784
Salmonella Harmelen	ATCC 15783
Salmonella Houton	ATCC 29834
Salmonella Illinois	ATCC 11646
Salmonella Indiana	CDC 343-86
Salmonella Infantis	CDC 139-84
Salmonella Inverness	CDC 133-87
Salmonella Iaviana	Silliker
Salmonella Jerusalem	Silliker
Salmonella Johannesburg	CDC 500-86
Salmonella Kentucky	Kentucky Food Company
Salmonella Kottbus	CDC 292-85
Salmonella Krefeld	CDC 939-84
Salmonella Livingstone	CDC 110-85
Salmonella Locarno	CDC 886
Salmonella London	CDC 382-84
Salmonella Madelia	CDC 288-85
Salmonella Malawi	CDC 327-80
Salmonella Manila	Manila Feed Company
Salmonella Maregrosso	CDC 235-77
Salmonella Mbandaka	Silliker
Salmonella Miami	CDC 114-84
Salmonella Michigan	CDC 47-85
Salmonella Minnesota	CDC 245-86
Salmonella Montevideo	Silliker
Salmonella Muenchen	CDC 427-85
Salmonella Newbrunswick	Silliker
Salmonella Newport	CDC 520-86
Salmonella Nienstedten	Silliker
Salmonella Ohio	CDC 299-86
Salmonella Oranienburg	CDC 447-86
Salmonella Ouakam	CDC 32-87
Salmonella Panama	CDC 551-86
Salmonella Paratyphi A	CDC 1072-82
Salmonella Paratyphi B	ATCC 8759
Salmonella Paratyphi C	ATCC 13428
Salmonella Phoenix	CDC 696-84
Salmonella Poona	CDC 251-86
Salmonella Potsdam	ATCC 25957
Salmonella Putten	ATCC 15787
Salmonella Rissen	Silliker
Salmonella Roodeport	Roodepoort Pet Food Company
Salmonella Rubislaw	Silliker
Salmonella Saintpaul	Silliker
Salmonella Salamae	ATCC 15786
Salmonella Sandiego	ATCC 23199

Genus serotypes	Source‡
Salmonella Saphra	CDC 479-85
Salmonella Schwarzengrund	Schwarzengrund Feed Company
Salmonella Senftenberg	Silliker
Salmonella Simsbury	ATCC 12004
Salmonella Sundsvall	CDC 668-84
Salmonella Tallahasee	ATCC 12002
Salmonella Tennesse	Tennesse Bakery
Salmonella Thompson	Thompson Food Company
Salmonella Tranora	CDC 857
Salmonella Treforest	CDC 657
Salmonella Typhi	CDC 2049-87
Salmonella Typhimurium	ATCC 6994
Salmonella Urbana	Silliker
Salmonella Vellore	ATCC 15611
Salmonella Wassenaar	CDC 121-87
Salmonella Weltevreden	CDC 110-83
Salmonella Weslasco	CDC 185-87
Salmonella Worthington	CDC 247-85

‡ NCTC: National Collection of Type Cultures, USA; ATCC: American Type Culture Collection, USA; Silliker: Silliker Food Science Center Culture Collection, USA: CDC: Centers for Disease Control, USA.

Exclusivity

Genus serotypes	Source‡
Acetobacter aceti	ATCC 23746
Citrobacter amalonaticus	Testing Kit Company
Citrobacter farmieri	ATCC 51112
Citrobacter freundii	ATCC 6879
Citrobacter braakii	ATCC 29063
Citrobacter youngae	ATCC 29935
Enterobacter aerogenes	ATCC 35028
Enterobacter agglomerans	ATCC 29917
Enterobacter clocae	ATCC 35030
Enterobacter clocae	ATCC 7256
Enterobacter sakazakii	ATCC 51329
Enterococcus faecalis	ATCC 49452
Enterococcus faecalis	ATCC 33186
Escherichia blattae	ATCC 29907
Escherichia coli	ATCC 11229
Escherichia coli 0157:H7	ATCC 35150
Hafnia alvei	ATCC 25927
Klebsiella oxytoca	ATCC 13182
Klebsiella pneumoniae	ATCC 9591
Morganella morganii subsp. *Morganii*	ATCC 29853

Genus serotypes	Source‡
Providencia stuartii	Testing Kit Company
Proteus mirabilis	ATCC 25933
Proteus vulgaris	ATCC 8427
Serratia liquefaciens	ATCC 27592
Serratia marcescens	ATCC 14041
Shigella boydii	ATCC 9207
Shigella dysenteriae	ATCC 11456b
Shigella flexineri	ATCC 9199
Shigella sonneii	ATCC 25931
Staphylococcus aureus	ATCC 13565
Yersinia enterocolitica	ATCC 29913
Yersinia frederksensii	Silliker
Yersinia pseudotuberculosis	Testing Kit Company

‡ ATCC: American Type Culture Collection, USA; Silliker: Silliker Food Science Center Culture Collection, USA; CDC: Centers for Disease Control, USA.

B Troubleshooting

Observation	Possible cause	Recommended action
No IPC or target-specific signal is detected in unknown wells.	Inhibition of PCR occurred.	Repeat sample preparation, then repeat assay. If PCR is still inhibited, dilute the sample (for example, 1:5 or 1:10) to dilute inhibitors. Alternatively, use a Bacterial Genomic DNA Purification Kit (Major Laboratory Supplier) to remove inhibitors.
	Environmental Master Mix not stored properly	Repeat the assay using properly stored assay components. Avoid freezing and thawing assay components. Protect the Assay Mix from light.
	Target-specific 10X Assay Mix not stored properly	
	Pipetting error (no premix solution added)	Repeat the assay. Make sure to pipette premix solution into all wells.
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.
No IPC signal is detected, but target-specific signal is detected in negative control wells.	Carryover contamination and one of the following: • 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 unknown wells.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.	No action required.
No IPC or target-specific signal is detected in positive control wells.	• Environmental Master Mix was not stored properly. • Target specific 10X Assay Mix not stored properly.	Repeat the assay using properly stored assay components. Avoid freezing and thawing assay components. Protect the Assay Mix from light.

Observation	Possible cause	Recommended action
No target-specific signal is detected in positive control wells.	A pipetting error occurred (no positive control added).	Repeat the assay. Make sure to pipet the positive control into all positive control wells.
Target-specific signal is detected in negative control well.	Carryover contamination occurred.	Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. If the negative control still shows contamination, repeat the assay using a new kit. If the negative control continues to show contamination, contact Life Technologies Technical Support.
Replicate results for this sample are inconsistent.	All replicate wells for a sample do not have the same result. Replicates may have positive, negative, and/or warning results.	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.

C

References

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D

Good PCR Practices

Prevent contamination and nonspecific amplification

Overview

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

PCR good laboratory practices

When preparing samples for PCR amplification:

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

  Note: Rooms can be simulated using a clean bench or PCR bench available from major laboratory suppliers.
- 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 and before leaving the work area.
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes and reaction plates carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use positive-displacement pipettes or aerosol-resistant pipette tips.
- Clean lab benches and equipment after use with 10% bleach solution, followed by a 70% ethanol rinse to remove residual bleach.

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.

Plate layout suggestions

- Separate different targets by a row if enough space is available.
- Put at least one well between unknown samples and controls if possible.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- Put positive controls in one of the outer rows or columns if possible.
- Consider that caps come in strips of 8 or 12.

E

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, etc). To obtain SDSs, see "Obtaining SDSs" on page 33.

Chemical safety

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

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see "Obtaining SDSs" on page 33.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

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 using the instrument.

WARNING! CHEMICAL HAZARD. CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. 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.

Specific chemical handling

WARNING! CHEMICAL HAZARD. MicroSEQ® *Salmonella enterica* Detection Kit. 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, mouth, and skin. Wear appropriate protective eyewear, clothing, and gloves.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

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

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Food safety support

Website: **www.lifetechnologies.com/foodsafety**

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

Phone number (Inside North America): 1-800-500-6885

Phone number (Outside North America): Go to **www.lifetechnologies.com/contactus.html** and select the appropriate country from the drop-down menu.

Limited product warranty

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

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