

MBPCR273

Hi-PCR® Salmonella Food Probe PCR Kit

Description

Salmonella contamination in food production and processing is a serious threat as it affects the intestinal tract of the consumer. Salmonella infection (salmonellosis) is a common bacterial disease that affects the intestinal tract. Humans become infected most frequently through contaminated water or food. Typically, people with salmonella infection have no symptoms. Others develop diarrhea, fever and abdominal cramps within 8 to 72 hours. Most healthy people recover within a few days without specific treatment. In some cases, the diarrhea associated with salmonella infection can be so dehydrating as to require prompt medical attention. Life-threatening complications also may develop if the infection spreads beyond your intestines. Your risk of acquiring salmonella infection is higher if you travel to countries with poor sanitation. Nucleic acid amplification-based assays or Polymerase Chain Reaction (PCR) is an alternative method that allows for sensitive and specific detection of Salmonella species from clinical samples / cultured isolates. Real-Time PCR technique is considerably simple and fast with respect to the standard PCR technique. This technique has been successfully used for the rapid detection and identification of a variety of infectious and non-infectious pathogens and genes.

NOTE: HiMedia's Hi-PCR® Salmonella Food Probe PCR Kit is for *in vitro* use only.

Intended Use

Hi-PCR® Salmonella Food Probe PCR Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR. The kit is recommended for sensitive and specific detection of Salmonella. The Hi-PCR® Salmonella Food Probe PCR Kit targets 100% of the known Salmonella strains.

Product Description:

Hi-PCR® Salmonella Food Probe PCR Kit is based on real-time PCR technology for the detection of Salmonella DNA. The assay principle is based on hydrolysis probe chemistry which confers higher specificity and sensitivity.

Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to ensure proper and intended functioning of all the reagents and is recommended to be used in every run to assess optimal performance.

Internal Control

This is a control sequence which is amplified in the same reaction tube along with the target sequence (target species) but detected with a different primer (i.e. Multiplex PCR). An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

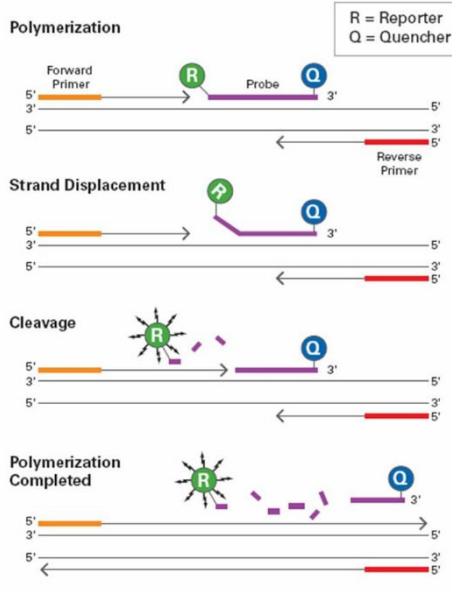
Negative Control

A Negative control is needed to ensure that the reagents, equipment, and environment used in the assay is not contaminated. In this reaction, Nuclease free water is used as the template. It is recommended to have minimum 1 reaction of negative control per run.

Principle

Real-Time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Hi-PCR® Salmonella Food Probe PCR Kit is designed to specifically detect **Salmonella DNA in FAM channel with Internal Control (IC) in JOE/HEX/VIC channel.**

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively.

Strand displacement: When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe.

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence.

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'-nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

- Highly sensitive and specific
- Includes all reagents & controls for validity of the test
- Fast – results within 2hrs

Types of Specimens and storage:

The internal validation of the HiMedia's Hi-PCR® Salmonella Food Probe PCR Kit was performed using DNA extracted from food samples. Other sample materials are not validated. After extraction, store the extracted DNA samples at -20°C for short period storage and -70°C or -80°C for long period storage.

Specimen Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with other body fluids. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C to -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity of the assay. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on pack.

Kit Contents

The provided PCR kit contains:

Components	Product code	Reagents provided for reactions (µL)		
		25R	50R	100R
Hi-Quanti 2X Realtime PCR Master Mix	MBT180	270 µL	540 µL	1060 µL
Salmonella spp. primer probe mix	DS1632	54 µL	108 µL	212 µL
Internal Control primer probe mix	DS0630	54 µL	108 µL	212 µL
Positive control (Salmonella spp. DNA)	DS0357	25 µL	50 µL	100 µL
Internal Control DNA	DS1022C	27 µL	54 µL	106 µL
Molecular Biology Grade Water for PCR	ML065	25 µL	50 µL	100 µL

* For a 20 µL PCR reaction

Materials needed but not provided

All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q96® AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96® AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
Insta Q48® M4 Real time PCR System, 96 well block, 4 channels	LA1023
Insta Q48® M2 Real time PCR System, 96 well block, 2 channels	LA1024
TabSpin™ Microcentrifuge	LA1089/LA1090
Automated nucleic acid extraction system and materials	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag24	MBLA020
Extraction Kits	
HiPurA® Pre-filled Cartridges for Food Pathogen Purification	MB568MPF-24
Tubes, plates and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615

µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18

Kit compatibility with Real-Time PCR Systems

Hi-PCR® Salmonella Food Probe PCR Kit contains fluorophores that are compatible to the following PCR systems:

Real-Time PCR system	Company	Dye 1 (Salmonella)	Dye 2 (IC)
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	JOE
QuantStudio™ 3 and 5	Applied Biosystems	FAM	VIC
Applied Biosystems 7500	Applied Biosystems	FAM	JOE
BioRad CFX Opus 96/CFX96	Bio-Rad Laboratories, Inc.	FAM	HEX
Rotor-Gene® Q/QIAquant	QIAGEN	Green	Yellow
Roche LightCycler® 96	Roche	FAM	HEX
AriaMx	Agilent	FAM	HEX
Alta RT-96/48	Athenese-Dx Private Limited	FAM	JOE
qTOWER ³ auto	Analytik Jena	FAM	JOE

Note: Ensure that the Real-Time PCR system is calibrated for dyes and is maintained according to the manufacturer's instructions and recommendations

Warning and Precautions

Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

General Preparation Instructions

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

Protocol for PCR Master Mix Preparation

1. In the “Master mix Preparation” area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for several seconds. Keep on ice for later use.
2. Based on the number of specimens to be tested (N), including the quantitative standards and NC, calculate the volume of the components to be added as N* volume of 1X

Components	Volume to be added for 1R (20 µL reaction)
2X PCR TaqMixture	10 µL
Salmonella spp. primer probe mix	2 µL
Internal Control primer probe mix	2 µL
Internal Control DNA	1 µL
Template DNA/Negative Control/Positive Control	5 µL
Total volume	20 µL

3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the reaction system. After all the reagents are added, mix them thoroughly and centrifuge for several seconds.
4. Load 15 µL of master mix into the 0.1/0.2 mL PCR reaction tube/plate/strips, compatible to the instrument to be used; add 5 µL Nuclease free water to the negative control.
5. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
6. Briefly, spin the strips/tubes to settle the reagent to the bottom of the tube.
7. Place the plate/strips/tubes in Real-time PCR machine and set the PCR program.

Recommended PCR program

Step	Temperature	Time	Sampling	Cycles
1	95°C	10 minutes	---	1
2	95°C	15 seconds	---	40
3	50°C	30 seconds	Yes	

Selection of channel

Target	Dye	Quencher
Salmonella	FAM	None
Internal Control	JOE/HEX/VIC	None

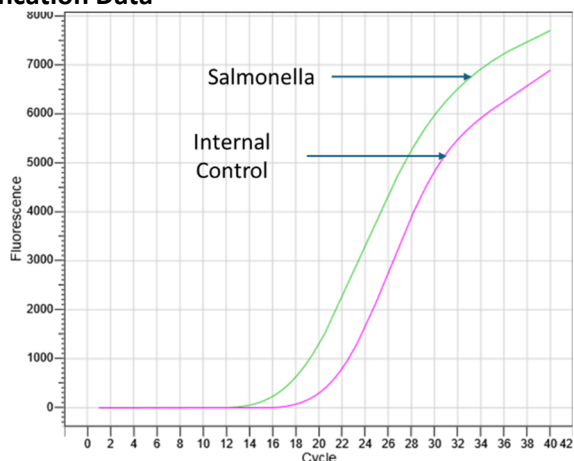
Passive Reference Dye: Select “None”

Data Analysis

The following conditions should be met for a valid diagnostic test:

Control	Detection channel	
	FAM (Salmonella)	JOE/HEX/VIC (Internal Control)
Positive Control	+	+
Negative Control	-	+

Amplification Data



Sr. No.	Target	Ct value	
		PC	NC
1	Salmonella	18.29	-
2	Internal Control	21.7	21.96

Image representing amplification plot of Salmonella using HiMedia's Hi-PCR® Salmonella Food Probe PCR Kit. The results completely depend upon sample types

Data Interpretation

Ct Value	Result
≤ 35	Detected (+)
> 35	Not detected (-)

Target		Result Interpretation
Salmonella spp. (FAM)	Internal Control (JOE/HEX/VIC)	
+	+/-*	Positive for Salmonella spp.
-	+	Negative for Salmonella spp.
-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

*The presence or absence of a signal in the JOE channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

Analytical Performance

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® Salmonella Food Probe PCR Kit was conducted using food specimens on InstaQ96® Real Time PCR system and Bio-Rad Opus Real Time PCR system. The detectable limit of the Hi-PCR® Salmonella Food Probe PCR Kit on both instruments was determined to be 10 cfu/mL.

Cross-reactivity - Analytical Specificity

In silico analysis was performed using NCBI nucleotide and Primer BLAST. The primers for Hi-PCR® Salmonella Food Probe PCR Kit were analyzed against bacteria, yeast and viruses. The Hi-PCR® Salmonella Food Probe PCR Kit was 100% specific for the known Salmonella strains.

Evaluation

Each lot of Hi-PCR® Salmonella Food Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® Salmonella Food Probe PCR Kit is functionally tested in amplification assay.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Check the integrity of DNA using agarose gel electrophoresis. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Check whether all components are added in correct volume as per the manual.
		Inappropriate storage conditions	Store the reagents at recommended temperature for its optimal performance. Check expiry of the reagents and use new lot of reagents if necessary.
		Incorrect PCR programming	Ensure selection of appropriate fluorescence channel as detailed in the manual Compare the PCR program to the manual.
2.	Variability between replicates	Error in reaction set-up	Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a Real-Time PCR instrument.
		Pipetting error	C _t values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes. Use calibrated pipettes. Repeat the run.
3.	Amplification in negative template control	Cross contamination during handling	Replace all critical solutions. Repeat the analysis of all tests with fresh aliquots of critical reagents. Follow good laboratory practices to avoid contamination issues.
4.	Standard Curve does not show expected PCR parameters such as efficiency and slope	Improper Mixing of standards	Thaw and mix the standard vials properly before putting the PCR run.
		Inappropriate storage conditions	Check the storage condition and

Safety Information

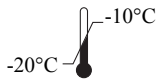
HiMedia's Hi-PCR® Salmonella Food Probe PCR Kit for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



Storage temperature



Do not use if package is damaged



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