

## MBPCR196

## Hi-PCR® Salmonella Quantification Probe PCR Kit

### Instructions For Use

#### Description

*Salmonella* infection (salmonellosis) is a common bacterial disease that affects the intestinal tract. *Salmonella* bacteria typically live in animal and human intestines and are shed through feces. 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 eight 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 quantification of *Salmonella* spp. 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 Quantification Probe PCR Kit is for *in-vitro* use only.

#### Intended Use

Recommended for sensitive and specific quantification of *Salmonella* spp. in clinical and environmental samples.

#### 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 Quantification Probe PCR Kit is designed to detect the **ompF region of Salmonella in FAM channel with Internal Control in JOE channel** in a single tube reaction. The kit allows sensitive and specific quantification of *Salmonella* spp. in a single tube reaction.

#### Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to check that the primers have been designed properly and the PCR conditions have been set up correctly.

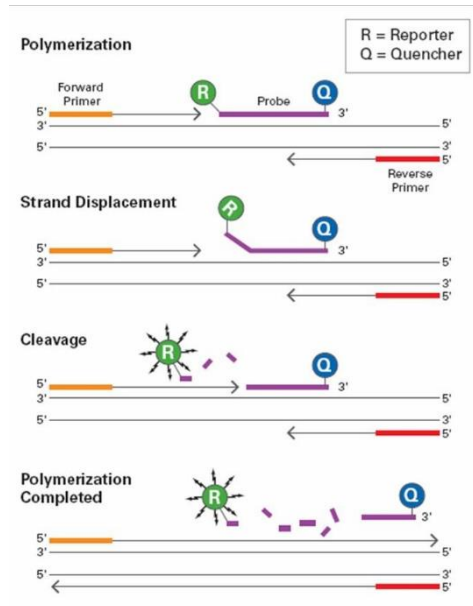
#### Internal control

This is a control sequence which is amplified in the same reaction tube along with the target sequence (target pathogen) 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, Molecular Biology Grade Water is used as the template. It is recommended to have minimum 1 reaction of negative control per run.

## 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

- Fast and simple
- Good sensitivity and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

**Types of Specimen:** Blood sample / bacterial cultures / Food samples/ Water samples

## Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored at -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. 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	
		25 reactions* (µL)	50 reactions* (µL)
2X Super Mastermix	DS0900	338	675
<i>Salmonella</i> spp. Primer-Probe Mix	DS0422	27	54
Internal Control Primer-Probe Mix	DS1117	27	54
Internal Control DNA	DS1096	27	54
Molecular Biology Grade Water for PCR	ML065	200	400
<i>Salmonella</i> Standard DNA 1	DS0871	13	25
<i>Salmonella</i> Standard DNA 2	DS0872	13	25
<i>Salmonella</i> Standard DNA 3	DS0873	13	25
<i>Salmonella</i> Standard DNA 4	DS0874	13	25

\* For a 25 µL PCR reaction

### Specimen collection and 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 blood and other body fluids. Safety guidelines may be referred in individual safety data sheets.

### Sample Preparation

Various clinical samples / cultured isolates are routinely examined. For extraction and purification of pure DNA for high yield, perform the nucleic acid purification using HiMedia's extraction kits as instructed in the protocol.

### Materials needed but not provided

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19) & Sealing film (PR18)
- Insta Q Real Time PCR System (Product Code: LA1012 / LA1023 / LA1024 / LA1073 / LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes
- For clinical samples: HiPurA® Multi-Sample DNA Purification Kit (MB554)
- For cultured isolates: HiPurA® Bacterial Genomic DNA Purification Kit (MB505)
- For Food samples: HiPurA® Food Pathogen DNA Purification Kit (Salmonella) [Provided with culture media] (MB568) / HiPurA® Food Pathogen (Bacteria) DNA Purification Kit (MB568A) / HiPurA® Food DNA Purification Kit (MB562)
- For Water samples: HiPurA® Water DNA Purification Kit (with enrichment) (MB547) and HiPurA® Water DNA Purification Kit (without enrichment) (MB577)

### 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.

### A. Protocol for PCR Master Mix Preparation

Components	Product Code	Volume to be added for 1R (for a 25 µL reaction)
2X Super Mastermix	DS0900	12.5 µL
<i>Salmonella</i> spp. Primer-Probe Mix	DS0422	1 µL
Internal Control Primer-Probe Mix	DS1117	1 µL
Internal Control DNA	DS1096	1 µL
Molecular Biology Grade Water for PCR	ML065	4.5 µL
Standard DNA / Template DNA / Negative Control	-	5 µL
Total volume	-	Upto 25 µL

Centrifuge the tube briefly at 6000 rpm for about 10 seconds. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

### B. Recommended PCR program

- |                          |                       |                     |
|--------------------------|-----------------------|---------------------|
| 1. Initial denaturation  | : 95°C for 10 minutes | } No. of cycles: 40 |
| 2. Denaturation          | : 95°C for 15 seconds |                     |
| 3. Annealing & Extension | : 60°C for 30 seconds |                     |
| Sampling                 | : FAM/JOE             |                     |
| 4. Hold                  | : 4°C for ∞           |                     |

### Data Analysis

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

Control	Detection channel	
	FAM ( <i>Salmonella</i> spp.)	JOE (Internal Control)
Positive Control	+	+
Negative Control	-	+

### Data Interpretation

Detection Channel		Result Interpretation
FAM ( <i>Salmonella</i> spp.)	JOE (Internal Control)	
+ (≤ 35 Ct)	+ (≤ 35 Ct) / No Ct or > 35*	Positive for <i>Salmonella</i> spp.
No Ct or > 35	+ (≤ 35 Ct)	Negative for <i>Salmonella</i> spp.
No Ct or > 35	No Ct or > 35	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.

### Standards used

Standard	Concentration (ng/µL)	Number of copies
Standard 1	5	900000
Standard 2	0.5	90000
Standard 3	0.05	9000
Standard 4	0.005	900

**Sensitivity: 1 copy**

### Quality Control

Each lot of HiMedia's Hi-PCR® Salmonella Quantification Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification. Each lot of HiMedia's Hi-PCR® Salmonella Quantification Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

### Warning

Not for Medicinal Use.

### Precautions

Read the procedure carefully before starting the experiment. 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.

### Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of DNA using agarose gel electrophoresis. 2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
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 <sub>t</sub> values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.
4.	No signal with positive controls	Incorrect programming of the temperature profile of the thermocycler	Compare the temperature profile to the manual.

### Safety Information

HiMedia's Hi-PCR® Salmonella Quantification Probe PCR Kit is 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 techniques.




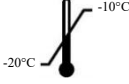







### Technical Assistance

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](mailto:mb@himedialabs.com).


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Please refer disclaimer Overleaf.

**Symbols:**

	Manufacturer		Do not use if package is damaged
	Authorized representative in the European Community		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		In vitro diagnostic medical device
	Batch code		CE marking of conformity
	Catalogue number		

**Authorized representative (AR) Address :**

	<b>AR Experts B.V.</b> Boeingavenue 209, 1119 PD, Schiphol-Rijk, The Netherlands
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Identification No.: PIMBPCR196

Rev.No.: 09

Date of Issue: 2026-01

**Disclaimer :**

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