MBPCR132 Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit

Instructions For Use

Carbapenem-hydrolyzing β-lactamases have rapidly spread among Gram-negative bacteria, posing a serious threat in hospital and community settings. These enzymes degrade most β-lactam antibiotics and resist common β-lactamase inhibitors. Their genes, often on mobile genetic elements, facilitate resistance spread, making rapid identification crucial for containment. Carbapenemases are prevalent in Enterobacteriaceae and other Gram-negative bacteria, and identifying specific types helps prevent unnecessary use of last-resort antibiotics. Nucleic acid amplification methods, particularly real-time PCR, offer a fast, sensitive, and specific approach for detecting carbapenemase genes in clinical samples and cultured isolates.

NOTE: HiMedia's Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit is for *in-vitro* use only.

Intended Use
Recommended for sensitive and specific detection of KPC, NDM, VIM, IMP, OXA-23, OXA-48, OXA-51 and OXA-58 like in clinical 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® Carbapenemase Gene (Multiplex) Probe PCR Kit is designed to detect the specific regions of the genes encoding the carbapenemase enzymes. There are three master mixes in this kit where in master mix-1 **NDM, KPC, IMP and VIM are detected in FAM, HEX, Texas Red and Cy5 channels** respectively, while in master mix-2 **OXA-51, OXA-23, OXA-48 and OXA-58 in FAM, HEX, Texas Red and Cy5 channels** respectively. Internal **Control is detected in JOE channel in master mix 3.** The kit allows sensitive and specific detection of single and co-present carbapenemases encoding genes 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 heterologous control (Internal Control, IC) sequence that serves as the in-process control to identify possible PCR inhibition and to confirm the integrity of the reagents of the Kit. It determines the validity of the tests.

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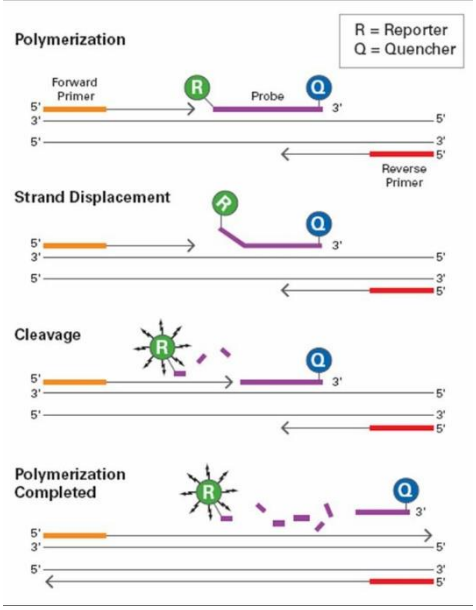
Negative Control

A Negative control is needed to ensure that the reagents, equipment, and environment used in the assay are not contaminated. In this reaction, Molecular Biology Grade Water is used as the template. It is recommended to have a minimum of 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 polymerae 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.

Molecular Features

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

Technology features:

- Fast and reliable results within 90 minutes.
- One-step assay i.e. reverse transcription and amplification are performed in same tube.
- Includes all reagents & controls for validity of the test.
- Open system – Compatible with 4-channel and 5-channel qPCR cyclers.
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

Sample type: Clinical samples / cultured isolates

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. 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 the pack.

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 to in individual safety data sheets.

Kit Contents:

The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)	
		25R	50R
CRG Master Mix	DS1915	1013	2025
CRG1 Primer-Probe Mix	DS2069	108	216
CRG2 Primer-Probe Mix	DS2070	108	216
Internal Control Primer-Probe Mix	DS1117	27	54
Internal Control DNA	DS1096C	27	54
CRG1 Positive Control Mix	DS0495	25	50
CRG2 Positive Control Mix	DS0496	25	50
Molecular Biology Grade Water for PCR	ML065	473	725

***** For a 25 µL PCR reaction

Materials needed but not provided

Appropriate real-time PCR instrument
Appropriate nucleic acid extraction system or kit
Centrifuge with a rotor for 1.5 mL - 2 mL reaction tubes
Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
Vortex mixer
PCR tubes (0.1ml or 0.2ml) or 96 well reaction plates with corresponding (optical) closing material or lid
Pipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)
Pipette tips with filters (As per pipette capacity)
Powder-free gloves (disposable)

All these 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® Mag16, Insta NX® Mag16^{Plus}	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32^{Plus}	LA1096, MBLA019
Insta NX® Mag96	LA1097
Extraction Kits	
HiPurA® Bacterial Genomic DNA Purification Kit	MB505
HiPurA® Multi-Sample DNA Purification Kit	MB554
HiPurA® Pre-filled Plates for Bacterial DNA Purification	MB505MPF-32
HiPurA® Pre-filled Plates for Food DNA Purification	MB505MPF16
HiPurA® Pre-filled Plates for Bacterial DNA Purification	MB505MPF-96
HiPurA® Pre-filled Cartridges for Bacterial DNA Purification	MB505PC16
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® Carbapenemase Gene (Multiplex) Probe PCR Kit contains fluorophores that are compatible to the following PCR systems. Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit however has been validated on Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

Real-Time PCR system	Company	Dye 1	Dye 2	Dye 3	Dye 4
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	HEX	Texas Red	Cy5
BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch	Bio-Rad Laboratories, Inc.	FAM	JOE/HEX	Texas Red	Cy5
QuantStudio™ 5	Applied Biosystems	FAM	JOE/HEX/VIC	Texas Red/ ROX	Cy5
ABI® Prism SDS 7500	Applied Biosystems	FAM	JOE/HEX/VIC	Texas Red/ ROX	Cy5
QIAquant 96 & 384 5plex	QIAGEN	FAM	JOE/HEX	Texas Red	Cy5
Rotor-Gene®6000 & Q	QIAGEN	FAM	JOE/HEX	Texas Red	Cy5
LightCycler® 96 / LightCycler® 480	Roche	FAM	JOE/HEX/VIC	ROX/ Texas Red	Cy5
qTOWER^3	Analytik Jena	FAM	JOE/HEX/VIC	ROX/ Texas Red	Cy5

Note: Ensure that the Real-Time PCR system is calibrated for dyes mentioned above and maintained according to the manufactureŕs instructions and recommendations.

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 5 seconds. Keep on ice for later use.
2. Based on the number of specimens to be tested (N), calculate the volume of the components to be added as N* volume of "1X"
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix of Tube 1, Tube 2 and Tube 3. Refer the following table. After all the reagents are added, mix them thoroughly and centrifuge for 5 seconds.

Components	Product Code	Volume to be added for 1R		
		CRG1 Tube	CRG2 Tube	IC Tube
CRG Master Mix	DS1915	12.5 µL	12.5 µL	12.5 µL
CRG1 Primer-Probe Mix	DS2069	4 µL	-	-
CRG2 Primer-Probe Mix	DS2070	-	4 µL	-
Internal Control Primer-Probe Mix	DS1117	-	-	1 µL
Internal Control DNA	DS1096C	-	-	1 µL
Molecular Biology Grade Water for PCR	ML065	3.5 µL	3.5 µL	5.5 µL
Positive Control / Negative Control / Template DNA	-	5 µL	5 µL	5 µL
Total volume	-	25 µL	25 µL	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).

Recommended PCR program

1.	Initial denaturation	:	95°C for 10 minutes	
2.	Denaturation	:	95°C for 05 seconds	
3.	Annealing and Extension	:	60°C for 60 secs (Plate Read)	No. of cycles: 45
	Sampling	:	FAM/HEX/JOE/Texas Red/Cy5	

Data Analysis

Selection of channels

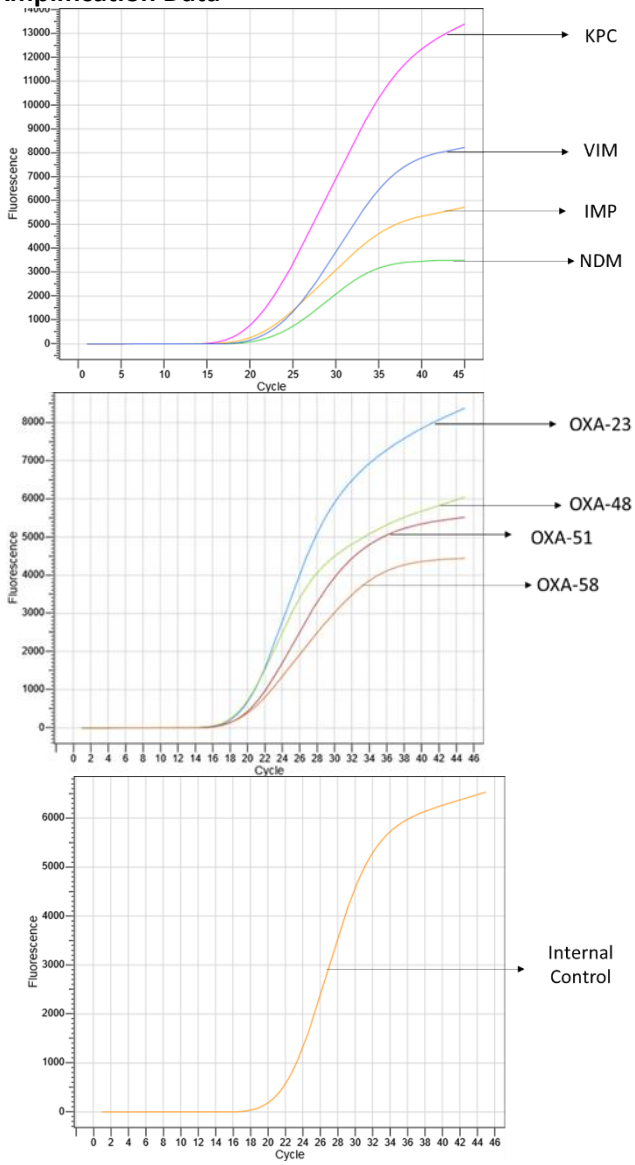
TUBE	Target	Channels	Quencher
CRG1 Tube	NDM	FAM	None
	KPC	HEX/JOE/VIC	None
	IMP	TexasRed/ROX	None
	VIM	Cy5	None
CRG2 Tube	OXA-51	FAM	None
	OXA-23	HEX/JOE/VIC	None
	OXA-48	TexasRed/ROX	None
	OXA-58	Cy5	None
IC Tube	Internal Control (IC)	HEX/JOE/VIC	None

Please select 'Passive reference dye' as 'None' wherever applicable

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

Control	CRG1 Tube				IC Tube
	NDM (FAM)	KPC (HEX)	IMP (Texas Red)	VIM (Cy5)	IC (JOE)
Positive Control	+	+	+	+	+
Negative Control	-	-	-	-	+
Control	**CRG2 Tube**				**IC Tube**
	OXA-51 (FAM)	OXA-23 (HEX)	OXA-48 (Texas Red)	OXA-58 (Cy5)	IC (JOE)
Positive Control	+	-	+	+	+
Negative Control	-	+	-	-	+

Amplification Data



Sr. No.	Sample	C_t value	
		PC	NC
1	NDM Positive control	23.64	-
2	KPC Positive control	20.71	-
3	IMP Positive control	21.26	-
4	VIM Positive control	23.65	-

Sr. No.	Sample	C_t value	
		PC	NC
1	OXA-23 Positive control	21.29	-
2	OXA-48 Positive control	21.94	-
3	OXA-51 Positive control	20.07	-
4	OXA-58 Positive control	21.65	-

Sr. No.	Sample	C_t value
1	Internal Control	22.74

Image representing amplification plot of NDM, KPC, IMP, VIM, OXA-23, OXA-48, OXA-5, OXA-58 and Internal Control with Ct values using HiMedia's Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit. The results completely depend upon sample types.

Data Interpretation

Ct value	Result
≤ 40	Detected (+)
> 41 or N/A	Not detected (-)

Target					Result Interpretation
CRG1 Tube				IC Tube	
NDM (FAM)	KPC (HEX)	IMP (Texas Red)	VIM (Cy5)	Internal Control (JOE)	
±	±	±	±	+	Carbapenemase resistant
-	-	-	-	+	Negative for Carbapenemase resistant
-	-	-	-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample
Target					Result Interpretation
CRG2 Tube				IC Tube	
OXA-51 (FAM)	OXA-23 (HEX)	OXA-48 (Texas Red)	OXA-58 (Cy5)	Internal Control (JOE)	
±	±	±	±	+	Carbapenemase resistant
-	-	-	-	+	Negative for Carbapenemase resistant
-	-	-	-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

Analytical Performance

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit was conducted using clinical specimens on InstaQ96® Real Time PCR system. The detectable limit of the Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit for each target was determined to be **< 10 copies/reaction** with co-amplification of all targets.

Inclusivity

In silico analysis for the assessment of inclusivity for the Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit was conducted by mapping the primers and probe against the available sequences in GenBank. The Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit targets 100% of the characterized strains.

Warning

Certified for *in vitro* Diagnostic Use (IVD). 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 to in the 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.

Performance and Evaluation

Each lot of HiMedia's Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

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_t 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

Hi-PCR® Carbapenemase Gene (Multiplex) 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.

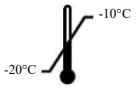
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Following established laboratory procedures in disposing of infectious materials and materials that comes into contact with clinical samples 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.

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 :

	**AR Experts B.V.** Boeingavenue 209, 1119 PD, Schiphol-Rijk, The Netherlands

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