

MBPCR209

Hi-PCR® Colistin Resistance Probe PCR Kit

Description

Colistin have recently regained popularity for treatment of severe infections caused by extensively drug-resistant (XDR) Gram-negative bacterial strains. As a likely consequence, emergence of Colistin resistance is being increasingly reported in the clinical setting, especially among carbapenem-resistant *Klebsiella pneumoniae* isolates. Acquired resistance to polymyxins is generally associated with chromosomal mutations. However, a new plasmid-mediated transferable resistance determinant, the *mcr-1* gene, which encodes for a phosphoethanolamine transferase, that alters the lipid A in the lipopolysaccharide has been described recently. The *mcr-1* gene was originally detected in Enterobacteriaceae (mostly *Escherichia coli*) of animal and human origin in China and subsequently also elsewhere, suggesting a broader distribution. Nucleic acid amplification-based assays or Polymerase Chain Reaction (PCR) is an alternative method that allows for sensitive and specific detection of *mcr-1* gene 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: Hi-PCR® Colistin Resistance Probe PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of colistin resistance by targeting the *mcr-1* gene.

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. Hi-PCR® Colistin Resistance Probe PCR Kit is designed to detect ***mcr-1* gene in FAM channel with Internal Control in JOE channel** in a single tube reaction. The kit allows sensitive and specific detection of *mcr-1* 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.

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.

Registered Office

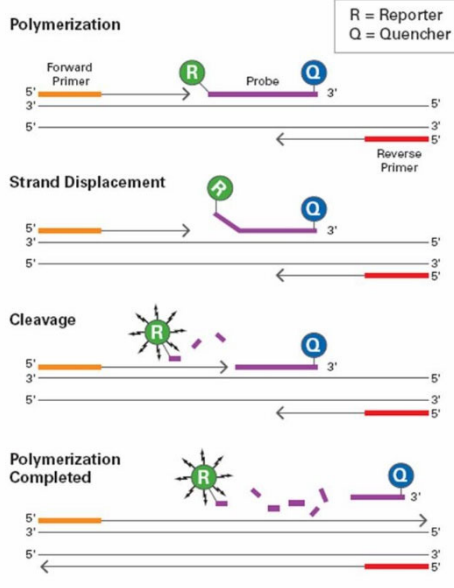
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Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



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

Sample Source: 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 pack.

Kit Contents: The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)	
		25R	50R
2X Super Mastermix	DS0900	338	675
<i>mcr-1</i> Primer-Probe Mix	DS0815A	27	54
Internal Control Primer-Probe Mix	DS0378A	27	54
Internal Control DNA	DS1096	27	54
<i>mcr-1</i> Positive Control	DS0816	25	50
Molecular Biology Grade Water for PCR	ML065	200	400

* 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 HiPurA® Multi-Sample DNA Purification Kit (MB554) for clinical samples and HiPurA® Bacterial Genomic DNA Purification Kit (MB505) for cultured isolates 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)

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

Perform PCR reactions for each DNA sample as per the following table:

Components	Product code	Volume (µL) to be added for 1R (for a 25 µL reaction)
2X Super Mastermix	DS0900	12.5 µL
<i>mcr-1</i> Primer-Probe Mix	DS0815A	1 µL
Internal Control Primer-Probe Mix	DS0378A	1 µL
Internal Control DNA	DS1096	1 µL
Molecular Biology Grade Water for PCR	ML065	4.5 µL
Template DNA / Positive Control / Negative Control	-	5 µL
Total volume	-	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

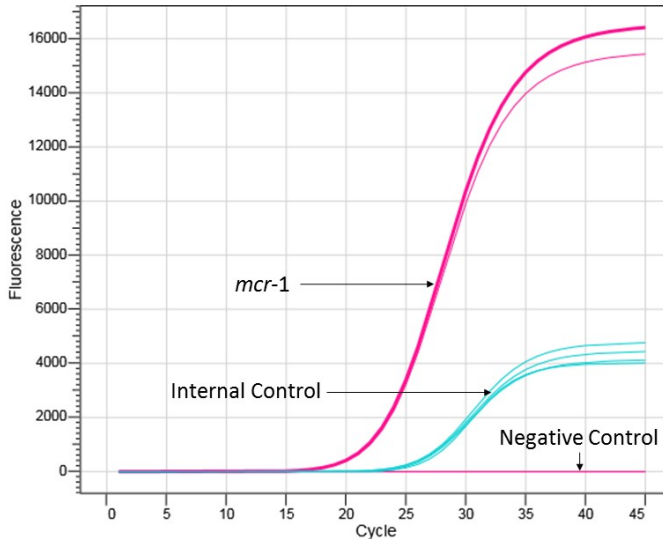
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|----------------------------|----------------------------------|---------------------|
| 1. Initial denaturation | : 95°C for 10 minutes | } No. of cycles: 45 |
| 2. Denaturation | : 95°C for 05 seconds | |
| 3. Annealing and Extension | : 60°C for 1 minute (Plate Read) | |
| Sampling | : FAM/JOE | |
| 4. Hold | : 4°C for ∞ | |

Data Analysis

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

Control	Targets	
	<i>mcr-1</i> (FAM)	Internal Control (JOE)
Positive Control	+	+
Negative Control	-	+

Amplification Data



Sr. No.	Sample	C _t value	
		PC	NC
1.	<i>mcr-1</i> Positive Control	22.26	--
2.	Internal Control	27.2	27.19

PC: Positive Control NC: Negative Control

Image representing amplification plot of *mcr-1* DNA with Ct values using Hi-PCR® Colistin Resistance Probe PCR Kit. The results completely depend upon sample types.

Data Interpretation

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

Detection Channel		
<i>mcr-1</i> (FAM)	Internal Control (JOE)	Result Interpretation
Ct value ≤ 40	Ct value ≤ 40*	Positive for <i>mcr-1</i>
Ct value >41	Ct value ≤ 40	Negative for <i>mcr-1</i>
No Ct	No Ct	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® Colistin Resistance Probe PCR Kit was conducted using clinical specimens on InstaQ96® Real Time PCR system. The detectable limit of the Hi-PCR® Colistin Resistance Probe PCR Kit for each target was determined to be **1 copies/reaction**.

Inclusivity

In silico analysis for the assessment of inclusivity for Hi-PCR® Colistin Resistance Probe PCR Kit was conducted by mapping the primers and probe against the available sequences in GenBank. Hi-PCR® Colistin Resistance 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 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.

Performance and Evaluation

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

Quality Control

Each lot of Hi-PCR® Colistin Resistance 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® Colistin Resistance 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

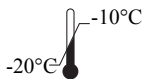
At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



In vitro diagnostic medical device



CE Marking



Storage temperature



Do not use if package is damaged



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01/2027

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