MBPCR261 Hi-PCR® Anthrax Multiplex Probe PCR Kit

Description:

Bacillus anthracis (*B. anthracis*), the causative organism of Anthrax is a Gram-positive spore forming, non-motile bacillus commonly found as endospores in soil. Anthrax is a zoonotic disease which is mainly associated with herbivores and domestic animals. Human anthrax is less common and usually spreads to human populations through close occupational proximity to infected livestock by handling infected domestic animals including cattle and goats or their products like skin, meat, hides and bones. The potential use of this bacterium as a bioterrorism agent has long been suspected because its spores can be aerosolized and sprayed to spread disease, are extremely resistant to natural conditions and can survive for several decades in the environment. *B. anthracis* spores enter the body through skin lesion (cutaneous anthrax), lungs (pulmonary anthrax), or gastrointestinal route (gastrointestinal anthrax) and germinate, giving rise to the vegetative form. Anthrax is a concern of public health also in many countries where agriculture is the main source of income including India. A rapid and sensitive method to detect *B. anthracis* is important for control of anthrax in animal cases to maintain public health and for appropriate treatment in human cases. Virulent strains of *B. anthracis* harbor two plasmids, pX01 and pX02, that carry unique genes conferring toxin production and capsule synthesis capability. PCR-based techniques have been successfully used for the rapid and accurate detection of virulent strains of *B. anthracis*.

NOTE: Hi-PCR® Anthrax Multiplex Probe PCR Kit is for *in vitro* use only.

Intended Use:

Hi-PCR® Anthrax Multiplex Probe PCR Kit is intended for use by qualified laboratory personnel trained in Real-Time PCR. The kit is recommended for sensitive and specific analysis of Bacillus anthracis 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. Hi-PCR® Anthrax (Multiplex) Probe PCR Kit is designed to detect a **genetic marker 5345 and two virulence genes located on plasmid pX01 and pX02 of Bacillus anthracis in FAM, ROX and Cy5 channel, respectively, and the Internal Control in JOE channel** in a single tube reaction. The kit allows sensitive and specific detection of Bacillus anthracis.

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.

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.

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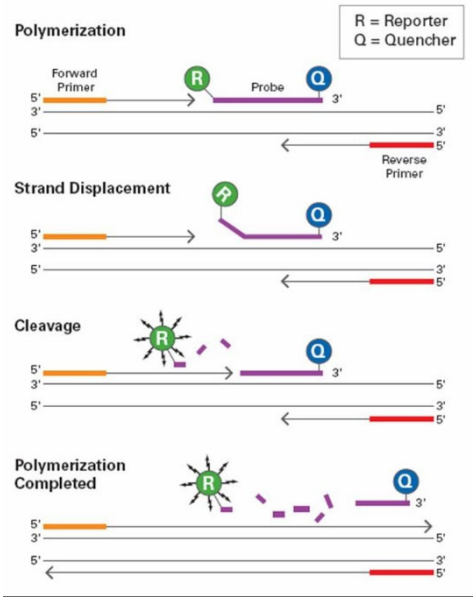
Internal Control

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.

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.

Features

- Fast and Simple – samples to results within 1.0 hours
- Good sensitivity and specific results
- Includes all reagents and controls
- Synthetic positive controls provided for validity of the test
- Guaranteed reproducible results

Sample Source: Blood, Plasma and Serum samples

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.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -15°C to -25°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
Anthrax Master Mix	DS1581	270	540
Anthrax Primer-Probe Mix	DS1582	27	54
Internal Control DNA	DS1583	27	54
Anthrax Positive Control	DS1584	40	80
Water	DS0440	40	80

*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® Real time PCR System, 96 well block, 5 channels	LA1012
Insta Q48® M4 Real time PCR System, 96 well block, 4 channels	LA1023
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
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
Kits and Reagents	
HiPurA® Viral DNA Purification Kit	MB575
Tubes, plates and other consumables	
Varivol II Micropipette-10 (Capacity: 0.5 to 10 µl)	LA611
Varivol II Micropipette-100 (Capacity: 10 to 100 µl)	LA615
Varivol II Micropipette-1000 (Capacity: 200 to 1000 µl)	LA614
Barrier Tips, Maximum capacity 10 µl	LA749/LA749A
Barrier Tips, Maximum capacity 200 µl	LA751/LA751A
Barrier Tips, Maximum capacity 1000 µl	LA859/LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.2 mL; PCR Plates	PW1255/ PR2/PR3/PR19
Optical Sealing film	PR18

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 (For one reaction)

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 PTC and NC, calculate the volume of the components to be added as N* volume of 1X

Components	Code	Volume for Test (for a 20 µL reaction)
Anthrax Master Mix	DS1581	10.0
Anthrax Primer-Probe Mix	DS1582	1.0
Internal Control DNA	DS1583	1.0
Molecular Biology Grade Water or Positive Control	ML065	8.0
Total volume		**20.0**

3. Load 12 µL of master mix into the 0.1/0.2 mL PCR reaction tube/plate/strips, compatible to the instrument to be used; add 12 µL of master mixture with 8 µL Nuclease free water to the negative control.
4. In the "Nucleic acid handling" area, add 1 µL internal control DNA in the positive and/or negative control reaction. Further, add 8 µL of the Positive Control and extracted test DNA into respective tubes in the plate/strip.
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.

B. Recommended PCR program

1.	Initial denaturation	:	95°C for 10 minutes	
2.	Denaturation	:	95°C for 15 seconds	No. of cycles: 40
3.	Annealing	:	60°C for 15 seconds (Plate Read)	
	Channel	:	FAM/ROX/Cy5/JOE	
4.	Hold	:	4°C for ∞	

Data Analysis

The following conditions should be met for a valid test:

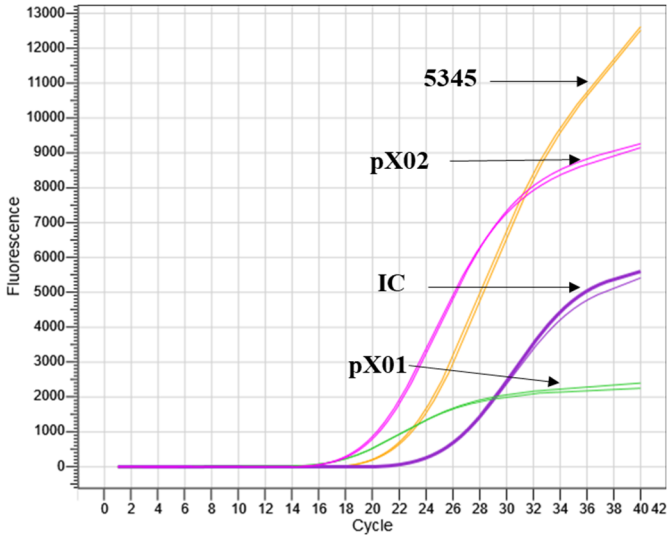
Control	Target			
	5345 (FAM)	pX01 (ROX)	pX02 (Cy5)	Internal Control (JOE)
Positive Template Control (PTC)	**+**	**+**	**+**	**+**
Negative Control (NC)	**-**	**-**	**-**	**+**

Data Interpretation:

Detection Channel				Result Interpretation
5345 (FAM)	pX01 (ROX)	pX02 (Cy5)	Internal Control (JOE)	
+	+	+	+	Positive for Anthrax
-	-	-	+	Negative for Anthrax
-	-	-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

Ct value	Result
≤ 35	Detected (+)
> 35 or N/A	Not detected (-)

Amplification Data



Sr. No	Sample	Ct value
1	5345	23.15
2	pX01	18.24
3	pX02	20.56
4	Internal Control	26.13

Representative image showing amplification plot of the three anthrax specific gene targets and the Internal Control with Ct values, using Hi-PCR® Anthrax Multiplex Probe PCR Kit. The results completely depend upon sample types.

Quality Control

Each lot of HiMedia's Hi-PCR® Anthrax Multiplex Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. It has been functionally tested in DNA amplification assays.

Analytical Performance

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® Anthrax Multiplex Probe PCR Kit was conducted using synthetic DNA of *Bacillus anthracis* 5345, *Bacillus anthracis* PX01 and *Bacillus anthracis* PX02 on InstaQ96® Real Time PCR systems. The detectable limit of the Hi-PCR® Anthrax Multiplex Probe PCR Kit on Real Time instrument was determined to be 56 copies/reaction for 5345 gene, 73 copies/reaction for PX01 gene and 62 copies/reaction for PX02 gene.

Inclusivity - Analytical Sensitivity

In silico analysis for the assessment of inclusivity for the Hi-PCR® Anthrax Multiplex Probe PCR Kit was conducted by mapping the primers and probes against all the available *Bacillus* species sequences in GenBank. The Hi-PCR® Anthrax Multiplex Probe PCR Kit targets 100% of the known *Bacillus anthracis* strains.

Cross-reactivity - Analytical Specificity

In silico analysis was performed using NCBI nucleotide and Primer BLAST. The primers used for Bacillus anthracis were analyzed against bacteria, yeast and virus and determined to be 100% specific. Wet testing analysis was performed against the following pathogens mentioned below in the table. No cross-reaction was observed with any strains.

Acinetobacter baumannii	*Clostridium perfringens*	*Shigellas* spp.
Bacillus cereus	Clostridium tetani	*Staphylococcus aureus*
Bacillus megaterium	*Coccidioides*	*Staphylococcus epiermidis*
Bacillus subtilis	*Enterrococcus faecalis*	*Vibrio cholerae*
Bacillus thuringiensis	*Escherichia coli*	Epstein-Barr Virus
Blastomyces dermatitdis	*Legionella pneumophila*	Hepatitis B Virus
Burkholderia pseudomallei	*Lesteria monocytogenes*	Hepatitis C Virus
Campylobacter jejuni	*Monilial vaginitis*	Hepatitis E Virus
Candida albicans	*Mycoplasma* spp.	Herpes Simplex Virus
Clostridium spp.	*Saccharomyces* spp.	Human Immunodeficiency Virus
Clostiridium botulinum	*Salmonella enterica*	

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.

Performance and Evaluation

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

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

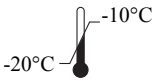
HiMedia's Hi-PCR® Anthrax Multiplex Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. 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.



Storage temperature

Do not use if package is damaged

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