Instructions for Use

MBPCR202 Hi-PCR® HLA-B27 Probe PCR Kit

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MBPCR202 **Hi-PCR® HLA-B27 Probe PCR Kit**

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1. Description

Human Leukocyte Antigen-B (HLA-B), as part of the major histocompatibility complex (MHC) class I, is involved in the recognition of self and foreign peptides. The allelic variant of Human Leukocyte Antigen (HLA) serotype B27 (HLA-B27) is present in about 8% of healthy Caucasians and is well known as genetic risk factor for spondyloarthropathies. Up to 96% of patients with ankylosing spondylitis (Morbus Bechterew) and up to 80% of patients with reactive arthritis carry the HLAB27 allele. Ankylosing spondylitis is a chronic, inflammatory arthritis that mainly affects spinal joints and the sacroiliac joint. Patients suffer from chronic pain and stiffness in the back and pelvis and, with progressing disease, often develop spinal fusion. The disease usually manifests between 15-30 years of age and is three times more prevalent in males than in females. Often a time period of 5-10 years elapses between early, nonspecific symptoms and a definite diagnosis. Nucleic acid amplification-based assays or Polymerase Chain Reaction (PCR) is an alternative method that allows for sensitive and specific detection of HLA-B27 DNA from clinical samples. 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 pathogens.

NOTE: HiMedia's Hi-PCR® HLA-B27 Probe PCR Kit is for *in-vitro* use only.

2. Intended Use

Recommended for sensitive and specific detection of HLA-B27 related to ankylosing spondylitis.

3. 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® HLA- B27 Probe PCR Kit is designed to detect HLA-B27 in FAM channel with Endogenous Internal Control in JOE channel in a single tube reaction.

4. Controls

- **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.

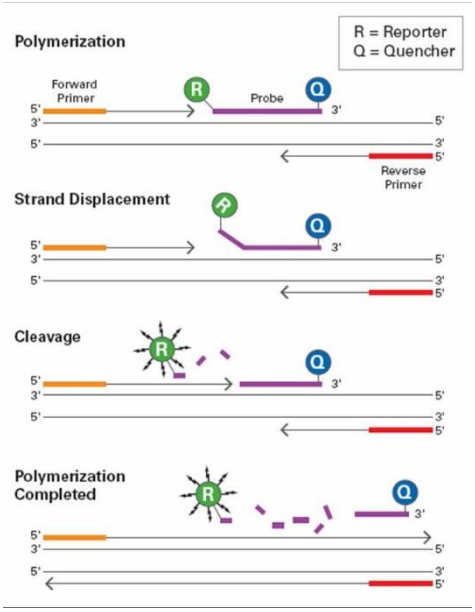
- **Negative Control**

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.

- **Internal Control**

This is a control sequence that should amplify in all clinical samples which indicates the presence of human endogenous gene. It 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.

5. Features

- Fast and simple
- Good sensitivity and specific results
- Guaranteed reproducible results

6. Types of Specimen

- DNA samples extracted from EDTA Blood samples using a standard blood DNA extraction kit.
- Before extraction, specimens can be stored at 4˚C up to 72 hours after collection. If any delay is expected in extraction, it is recommended to store specimens at -20 ˚C or lower.
- After extraction, store the extracted DNA samples at -20°C for short period storage and -70˚C or lower for long period storage.

7. 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 other body fluids.
- Safety guidelines may be referred in individual safety data sheets.

8. 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.
- This kit can be used for maximum 6 repeats of freezing and thawing.
- 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.

9. Kit Contents

Components	Product code	Reagents provided for (reactions)* (µL)	
		25R	50R
HLA-B27 Master Mix	DS1752	169	338
HLA-B27 Primer-Probe Mix	DS1185	54	108
HLA-B27 Positive Control	DS1178A	25	50
Molecular Biology Grade Water for PCR	ML065	318	635

***** For a 25 µL PCR reaction

10. Devices, Materials, and Reagents required 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® Mag16, Insta NX® Mag16^{Plus}	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32^{Plus}	LA1096, MBLA019
Insta NX® Mag96	LA1097
Extraction Kits	
HiPurA® Pre-filled Plates for Blood Genomic DNA Extraction	MB504MPF16
HiPurA® Pre-filled Plates for Blood Genomic DNA Extraction	MB504MPF-32
HiPurA® Pre-filled Plates for Blood Genomic DNA Extraction	MB504MPF-96
HiPurA® Pre-filled Cartridges for Blood Genomic DNA Extraction	MB504PC16
HiPurA® Multi-purpose Magnetic Nucleic Acid Purification kit	MB583MDL
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Plates)	MB583MPFDL16
HiPurA® Pre-filled Multi-purpose Magnetic Nucleic Acid Purification kit (Cartridges)	MB583PCDL16

HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit	MB583MPF96200
HiPurA® Pre-filled Clinical Multi-purpose Nucleic Acid Purification Kit	MB583MPF32200
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Plates)	MB583MPF16200
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Cartridges)	MB583PC16200
HiPurA® Pre-filled Multi-purpose Magnetic Nucleic Acid Purification Kit (Plates)	MB583MPFDL32
HiPurA® Pre-filled Multi-purpose Magnetic Nucleic Acid Purification kit	MB583MPFDL96
HiPurA® Clinical Multi-purpose Magnetic Nucleic Acid Purification Kit Sample	MB583M
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

11. Kit Compatibility with Real-Time PCR systems:

Hi-PCR® HLA-B27 Probe PCR Kit contains fluorophores compatible to:

Real-Time PCR system	Company	Dye 1 (HLAB27)	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 FAM dye, JOE/HEX dye and is maintained as according to the manufactureŕ s instructions and recommendations.

12. Warning and Precautions

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use. 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.

13. 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.

14. 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.

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

Components	Product code	Volume to be added for 1R (for a 25 µL reaction)
HLA-B27 Master Mix	DS1752	6.25 µL
HLA-B27 Primer-Probe Mix	DS1185	2 µL
Molecular Biology Grade Water for PCR	ML065	11.75 µL
Template DNA / Positive Control / Negative Control	-	5 µL
Total volume	-	25 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 20 µL of master mixture into the 0.1/0.2 mL PCR reaction plate/strips, compatible to the instrument to be used, add 5 µL of the negative control.
5. In the "Nucleic acid handling" area, add HLA-B27 Positive Control and extract test DNA into the plate/strip.
6. Tightly cap the strips or seal the plate using an optically clear adhesive film.
7. Briefly, spin the strips/tubes to settle the reagent to the bottom of the tube.
8. Place the plate/strips in Real-Time PCR machine and set the PCR program.

16. Recommended PCR program

1.	Initial denaturation	: 95°C for 10 minutes	
2.	Denaturation	: 95°C for 15 seconds	
3.	Annealing	: 56°C for 30 seconds (Sampling)	No. of cycles: 40
	Sampling	: FAM/JOE[#]	
4.	Hold	: 4°C for ∞	

[#]for instruments not calibrated for JOE, HEX/VIC can be used

17. Data Analysis

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

Control	Detection channel	
	HLA-B27 (FAM)	Internal Control (JOE)
Positive Template Control	+	+
Negative Template Control	-	-

18. Data Interpretation

Targets		Assay Interpretation
HLA-B27 (FAM)	Internal Control (JOE)	
+	(+) or (-)*	Positive for HLA-B27
-	+	Negative for HLA-B27
-	-	Invalid test Repeat extraction or obtain a new specimen

*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.

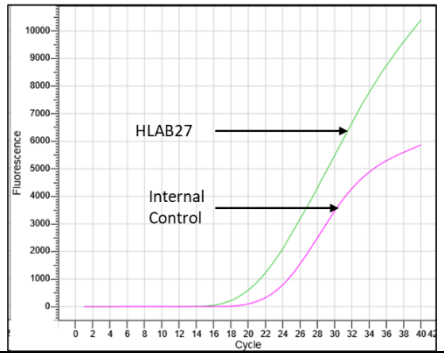
Ct value	Result
≤ 38	Detected (+)
> 38 or N/A	Not detected (-)

Note

- Positive results are indicative of the presence of HLA-B27 DNA. However, clinical correlation along with patient history is necessary to determine patient infection status. Positive results also do not rule out other infections/diseases.
- Negative results must be combined with clinical observations and patient history.

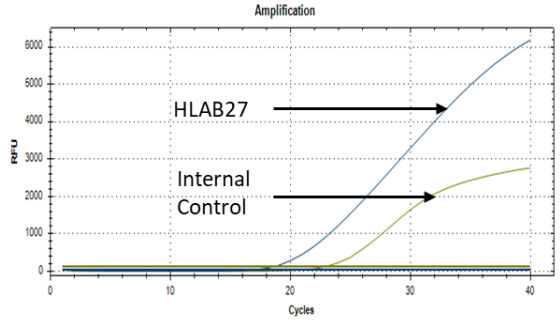
19. Amplification Data

InstaQ Series



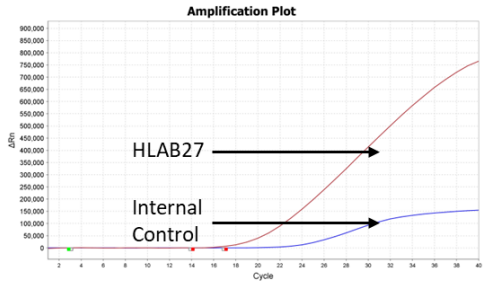
Target Name	Ct value
HLAB27 Positive Control	19.57
Internal Control Positive Control	22.07

Bio Rad CFX Opus



Target Name	Ct value
HLAB27 Positive Control	17.02
Internal Control Positive Control	23.02

QuantStudio 5



Target Name	Ct value
HLAB27 Positive Control	20.22
Internal Control Positive Control	24.17

Note: Image representing amplification plot of HLA-B27 Detection with Ct values using HiMedia's Hi-PCR® HLA-B27 Probe PCR Kit. (Ct values provided in table are for representation)

20. Performance Evaluation

➢ Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® HLA-B27 Probe PCR Kit was conducted using clinical specimens on InstaQ Series, Bio Rad CFX Opus and QuantStudio 5. The detectable limit of the Hi-PCR® HLA-B27 Probe PCR Kit was determined to be 1 copy/µL (5 copies/reaction).

➢ Inclusivity - Analytical Sensitivity

In-silico analysis for the assessment of inclusivity for the Hi-PCR® HLA-B27 Probe PCR Kit was conducted by mapping the primers and probes against all the available HLA-B27 sequences in GenBank. The Hi-PCR® HLA- B27 Probe PCR Kit targets 100% of the known HLA-B27 strains.

➢ Cross-reactivity - Analytical Specificity

In silico analysis was performed using NCBI nucleotide and Primer BLAST. The primers for HLA-B27 were analyzed against all organisms (data not shown because of large data set). Wet testing analysis was performed against the following pathogens. No cross-reaction was observed with any strains.

Wildtype *Escherichia coli*	Hepatitis C virus
Enterococcus faecalis	Hepatitis E virus
Klebsiella pneumoniae	Dengue
Acinetobacter baumannii	Hepatitis A virus
Staphylococcus aureus	*Plasmodium*
Pseudomonas aeruginosa	Human papillomavirus (HPV) 16
Streptococcus pneumoniae	Human papillomavirus (HPV) 18
Enterovirus	*Candida albicans*
Hepatitis B virus	

21. Evaluation

Each lot of Hi-PCR® HLA-B27 Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

22. Quality Control

Each lot of Hi-PCR® HLA-B27 Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non- specific DNase activities and has been functionally tested in amplification.

23. Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	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.	No amplification	Degraded samples	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.
4.	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.

24. Safety Information

Hi-PCR® HLA-B27 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.

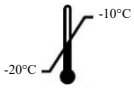
25. Disposal

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

26. Technical Assistance

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

27. Signs and 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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