

## MBPCR171

## Direct Blood PCR Kit

### Description

Researchers often purify DNA from blood samples prior to performing PCR because it is believed that blood constituents and the reagents commonly used to preserve blood samples (e.g., anticoagulants) interfere with PCR. In order to save the time and expense required for template purification, several methods have been reported for direct PCR of blood samples.

**NOTE: HiMedia's Direct Blood PCR Kit is for *in-vitro* use only.**

### Intended Use

The kit is designed for direct PCR from a wide range of human and animal whole blood samples.

### Principle

HiMedia's Direct Blood PCR Kit offers very fast, highly-specific, direct PCR from a wide range of human and animal whole blood samples, including those preserved with anticoagulants stored at different temperatures from +4°C, -20°C, and / or -70°C. The use of HiMedia's Taq polymerase enzyme has an increased affinity for template DNA, giving a high PCR product yield from the most challenging templates. The kit also allows fast cycling conditions to be used, without compromising PCR specificity and yield. This kit also contains universal 18S and GAPDH primers. A final concentration upto 10% can be used for Direct Blood PCR Kit. Specific primers can be used for identification of the target of interest by the end user. This kit also contain positive control.

### 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.

### Features

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

**Sample Source:** Blood samples

### 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 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 sensitivity of the assay. The kit provided is stable for 12 months when stored at mentioned conditions. HiMedia does not recommend using the kit after the expiry date stated on pack.

### Blood storage

For long term storage, it is recommended to store blood at -20 °C or dried on InstaDNA Cards (Product code: MBT126/MBT121/MBT137). For short term storage (less than 3 months), blood can be stored at 4 °C. EDTA, sodium citrate and heparin all work well with the HiMedia's Direct Blood PCR Kit.

1

Registered Office

**HiMedia Laboratories Pvt Ltd.**

Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA.  
Customer Care No.: 00-91-22-6116 9797  
Tel : 00-91-22-6147 1919, 6903 4800



Fax : 6147 1920

Web : [www.himedialabs.com](http://www.himedialabs.com)

Email : [info@himedialabs.com](mailto:info@himedialabs.com)

[mb@himedialabs.com](mailto:mb@himedialabs.com)

### The provided PCR kit contains

Components	Product codes	Reagents provided for (reactions)* (µL)	
		25R	100R
2X PCR TaqMixture	MBT061	675	1350
Primer Mix for 18S	DS0300	27	54
Primer Mix for GAPDH	DS0582	27	54
Positive control	DS0604	10	20
Molecular Biology Grade Water for PCR	ML065	550	1100

\* For a 50 µL 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

Fresh blood samples or samples preserved with anticoagulants stored at different temperatures from +4°C, -20°C, and / or -70°C can be used.

### 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)
- Thermal Cycler (Product Code: LA948 / LA949 / LA950 / LA1006 / LA1015 / LA1059 / LA1060 / LA1066)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes

### General Preparation Instructions

- Before use, suitable amount of all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control material (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.
- Centrifuge the components briefly once thawed.

### A. Protocol for PCR Master Mix Preparation

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

Components	Code	Recommended volume to be added per reaction
2X PCR TaqMixture	MBT061	25 µL
Primer Mix for 18S / Primer Mix for GAPDH	DS0300/ DS0582	0.1–1.0 µL
Blood sample	-	Upto 10%
Molecular Biology Grade Water for PCR	ML065	Up to 50 µL

Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below).

**NOTE: (Optional) – The user can also set up an additional PCR reaction containing 2µL of Positive control DNA (provided) in a separate tube.**

### B. Recommended PCR program

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation	94 or 95	5-10 mins	1
Denaturation	94 or 95	30 secs – 1 min	
Annealing	60	30 secs – 1 min	30-40
Extension	72	1 min/kb	
Final Extension	72	5 – 15 min	1

C. After amplification, the products may be kept at 4°C overnight or frozen at –20°C for long-term storage.

### D. PCR Assay Results Interpretation

- For analysis of the PCR data, load 10 µL of amplicon on a 1.5% Agarose gel along with 1 µL of 6X Gel Loading Buffer (ML015).
- Load 3 µL of 50 bp DNA ladder (MBT084) in separate well.

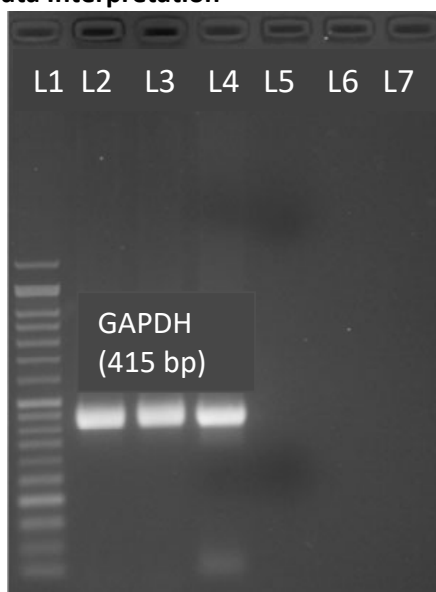
### E. EtBr-staining staining to check results

- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 minutes.
- Confirm the expected amplicon size comparing with 50 bp DNA marker.

### Quality Control

Each lot of HiMedia’s Direct Blood PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

### Data Interpretation



Lane no.	Samples
L1	50 bp ladder
L2 – L4	GAPDH PCR
L5- L7	Negative control

Gel image representing amplification of GAPDH gene (415bp)

### 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.

### Performance and Evaluation

Each lot of HiMedia’s Direct Blood 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 large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques 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.

### Safety Information

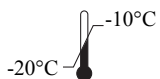
HiMedia's Direct Blood PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

### 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 to [mb@himedialabs.com](mailto:mb@himedialabs.com).



Storage temperature



Do not use if package is damaged



HiMedia Laboratories Private Limited,  
Reg. Off: Plot No. C-40, Road No. 21Y,  
MIDC, Wagle Industrial Estate, Thane,  
(West) 400604, Maharashtra, INDIA.  
Web: [www.himedialabs.com](http://www.himedialabs.com)



02/2027

PIMBPCR171\_O/0224

MBPCR171-06

**Disclaimer :**

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

HiMedia Laboratories Pvt. Ltd. Reg.office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA. Customer Care No.: 00-91-22-6116 9797 Tel: 00-91-22-6147 1919, 6903 4800 Email: [techhelp@himedialabs.com](mailto:techhelp@himedialabs.com) Website: [www.himedialabs.com](http://www.himedialabs.com)