

## **MBPCR124 Hi-PCR<sup>®</sup> Peste des petits ruminants (PPR) SYBr PCR Kit**

### **Description**

Peste des petits ruminants (PPR) are an acute or subacute viral disease of goats and sheep characterized by fever, necrotic stomatitis, gastroenteritis, pneumonia and sometimes death. The causal virus, a member of the Morbillivirus genus in the family Paramyxoviridae, preferentially replicates in lymphoid tissues and epithelial tissue of the GI and respiratory tracts, where it produces characteristic lesions. The genus Morbillivirus from the family Paramyxoviridae, a group of enveloped viruses with non-segmented, negative-strand RNA genomes is a virus that is highly infectious, spreads via the respiratory route and causes profound immune suppression. With animal healthcare becoming increasingly important, specific and faster methods for pathogen detection are the need of the hour.

**NOTE: Hi-PCR<sup>®</sup> Peste des petits ruminants (PPR) SYBr PCR Kit is for *in-vitro* use only.**

### **Intended Use:**

Recommended for sensitive and specific detection of morbillivirus in clinical samples.

### **Principle**

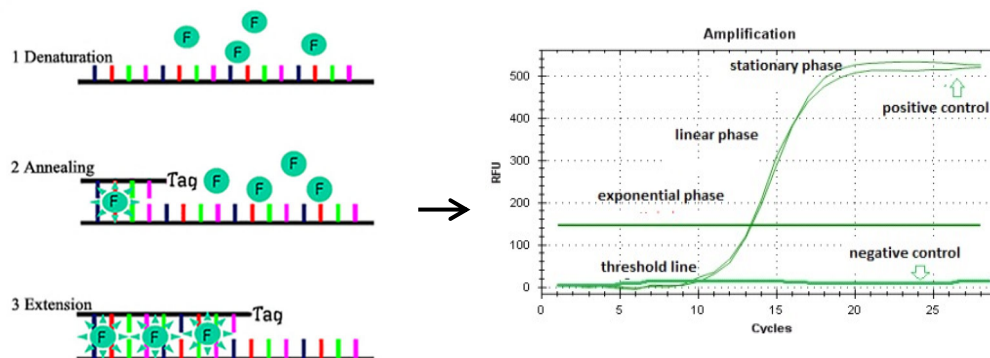
Hi-PCR<sup>®</sup> Peste des petits ruminants (PPR) SYBr PCR Kit is designed for detection of specific sequence of the large polymerase protein (L) gene giving amplification of 123 bp product of morbillivirus in clinical samples. This kit also contains Positive control.

### **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.

Real-time PCR also called quantitative PCR (qPCR) or kinetic PCR, is a laboratory technique based on the principle of PCR. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. The presence of SYBr Green Dye a dsDNA-binding dye in the Hi-SYBr Master Mix allows for simplified assay design without the need for additional fluorescent probes and enables assay verification using a melt-curve analysis. Hi-PCR<sup>®</sup> Peste des petits ruminants (PPR) SYBr PCR Kit are a qualitative Real-Time PCR kit which includes the amplification of L gene (123 bp) of morbillivirus.

## Diagrammatic representation of SYBr Green Chemistry in Real-Time PCR



The SYBr Green dye cycles between an unbound (Denaturation step) and a bound (Annealing through Extension) state as the reaction progresses. Signal intensity increases as the quantity of amplicons increase in later cycles indicating amplification. During elongation, more and more dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the dye molecules are released and the fluorescence signal falls.

### Features

- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results

**Sample Source:** Serum, tissue samples

### Storage and Shelf-life

The provided kit has a shelf-life of 12 months when stored between  $-10^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$ . 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 the sensitivity of the assay. HiMedia does not recommend using the kit after the expiry date stated on the pack.

### Kit Contents

The provided PCR Kit contains:

Components	Product Code	Reagents provided for (reactions)*	
		25R	50R
Hi-SYBr Master Mix (with Taq Polymerase)	MBT074	338 $\mu\text{L}$	675 $\mu\text{L}$
One Step RT Enzyme Mix	DS0286	27 $\mu\text{L}$	54 $\mu\text{L}$
PPR Primer Mix	DS0242A	41 $\mu\text{L}$	81 $\mu\text{L}$
PPR Positive Control	DS0271A	25 $\mu\text{L}$	50 $\mu\text{L}$
Molecular Biology Grade Water for PCR	ML065	160 $\mu\text{L}$	320 $\mu\text{L}$

\*For a 25  $\mu\text{L}$  PCR reaction

### 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
- HiPurA<sup>®</sup> Viral RNA Purification Kit (MB615)

### 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 to individual safety data sheets.

### General Preparation Instructions

- Before use, a 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 RNA/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 RNA sample as per the following table:

Components	Product Code	Recommended volume to be added per reaction (µL)
Hi-SYBr master mix (with Taq Polymerase)	MBT074	12.5
One Step RT Enzyme Mix	DS0286	1.0
PPR Primer Mix	DS0242A	1.5
Negative Control (ML065)/ Template (Extracted DNA) Positive Control (DS0271A)	-	5
Molecular Biology Grade Water for PCR	ML065	Up to 25

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).

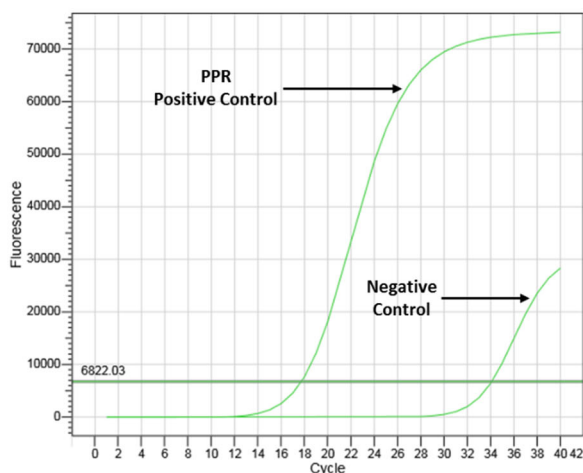
#### B. Recommended PCR program

1. Reverse Transcription : 50°C for 15 minutes
2. Initial denaturation : 95°C for 05 minutes No. of cycles: 1
3. Denaturation : 95°C for 10 seconds
4. Annealing : 55°C for 10 seconds } No. of cycles: 40
5. Melt Curve Analysis as per HiMedia's Insta Q96 Real Time PCR Machine
  - a. 95°C : 15 seconds
  - b. 60°C : 1 minute
  - c. 95°C : 15 seconds
  - d. Increment : 0.5°C
  - e. On Hold : 10 seconds

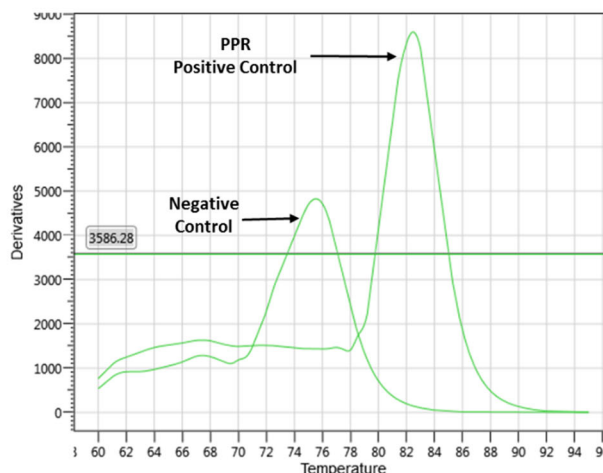
**NOTE: The user can also set up a melt curve program as per their existing PCR instrument.**

### C. Amplification data

**Amplification plot**



**Melt curve**



Sr. No.	Sample	C <sub>t</sub> value	T <sub>m</sub> Value
1	Positive control	17.7	80.3
2	Negative control	34.1	75.5

Image representing real-time amplification data of morbillivirus (C<sub>t</sub> and T<sub>m</sub> values provided in table)

#### Data Interpretation

Melting Temperature (T <sub>m</sub> )*	Result Interpretation
81°C-84°C	Positive for PPR

\* T<sub>m</sub> values can slightly vary for different sample types. If the T<sub>m</sub> values show significant variation from those mentioned in the above table, then the sample is considered to be negative for PPR.

#### Warning and Precautions

Not for Medicinal Use. 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 in safety data sheets of the product.

#### Performance and Evaluation

Each lot of Hi-PCR® Peste des petits ruminants (PPR) SYBr PCR Kit is tested against predetermined specifications to ensure consistent product quality.

#### Quality Control

Each lot of Hi-PCR® Peste des petits ruminants (PPR) SYBr PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested for amplification.

#### Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	<ol style="list-style-type: none"> <li>1. Check the integrity of RNA using agarose gel electrophoresis.</li> <li>2. Use freshly prepared RNA to ensure the availability of intact template sequence for efficient amplification.</li> </ol>

		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	<ol style="list-style-type: none"> <li>1. Replace all critical solutions.</li> <li>2. Repeat the analysis of all tests with fresh aliquots of critical reagents.</li> </ol>

### Safety Information

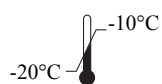
HiMedia's Animal Peste des petits ruminants (PPR) Detection Kit (Real-Time SYBr Based PCR) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

### Disposal

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



Storage temperature



Do not use if package is damaged



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### Disclaimer :

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