

MBPCR116 Hi-PCR® Peste des petits ruminants (PPR) Semi-Q 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® Peste des petits ruminants (PPR) Semi-Q PCR Kit is for *in-vitro* use only.

Intended Use:

Hi-PCR® Peste des petits ruminants (PPR) Semi-Q PCR Kit is a qualitative conventional PCR kit which results in amplification of PPR virus (PPRV) matrix gene. Conventional PCR testing can provide rapid, sensitive and specific detection of PPR.

Principle

Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of genes. The three steps of a successful PCR reaction include Denaturation, Annealing and Extension. The double-stranded DNA melts and forms single stranded DNA at high temperature (Denaturation). Sequence specific primers bind to the target sequence on single-stranded DNA at lower temperature (Annealing). *Taq* DNA Polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (Extension). These 3 steps of PCR are usually repeated between 30 to 40 times in each PCR assay. Hi-PCR® Peste des petits ruminants (PPR) Semi-Q PCR Kit is a conventional PCR kit which includes the amplification of matrix gene (M) (124 bp) of PPRV.

Negative Control

A negative control is needed to ensure that the reagents, equipment and environment used in the assay are 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 check that the primers have been designed properly and the PCR conditions have been set up correctly.

Features

- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

Sample source: Serum, tissue from vital organs

Storage and Shelf-life

The provided kit has a shelf-life of 12 months when stored at 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 RNA/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)* (µL)	
		25R	50R
One step RT Buffer	DS0221	270	540
10X Solution H	DS0222	135	270
MMuLV Reverse Transcriptase	DS0220	54	108
PPR Primer Mix	DS0242A	81	162
PPR Positive Control	DS0271A	25	50
Molecular Biology Grade Water for PCR	ML065	500	1000
6X Gel Loading Buffer	ML015	54	108
50 bp DNA Ladder	MBT084	15	30

*For a 50 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 to individual safety data sheets.

Sample Preparation

Various clinical samples are routinely examined. For extraction and purification of pure Viral RNA for high yield, perform the nucleic acid purification using HiMedia's HiPurA® Viral RNA Purification Kit (MB615) 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)
- 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, 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	Recommended volume to be added per reaction (50 µL)
One step RT Buffer	10
10X Solution H	5
MMuLV Reverse Transcriptase	2
PPR Primer Mix	3
Test – Extracted Sample RNA Positive Control – Provided in Kit Negative Control - Water	5
Molecular Biology Grade Water for PCR	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).

B. Recommended PCR program

- | | | |
|--------------------------|------------------------|---------------------|
| 1. Reverse Transcription | : 50 °C for 15 minutes | No. of cycles: 1 |
| 2. Initial denaturation | : 95°C for 5 minutes | No. of cycles: 1 |
| 3. Denaturation | : 94°C for 15 secs | } No. of cycles: 30 |
| 4. Annealing | : 55°C for 30 secs | |
| 5. Extension | : 72°C for 30 secs | |
| 6. Final Extension | : 72°C for 5 minutes | No. of cycles: 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 2% Agarose gel along with 1 µL of 6X Gel Loading Buffer (ML015).
- Load 3 µL of 50 bp DNA ladder (MBT084) in separate well.

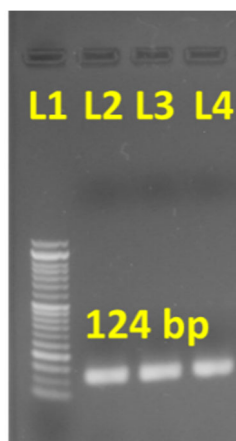
CI. EtBr-staining staining to check results

- 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 Hi-PCR® Peste des petits ruminants (PPR) Semi-Q PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific RNase / DNase activities. Functionally tested for amplification.

Amplification Data



Lane	Sample
L1	50 bp DNA Ladder
L2 to L4	Amplicon of PPRV (124 bp)

Gel image representing amplification of PPRV matrix gene; product size (124 bp)

Precautions

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) Semi-Q 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 RNA using agarose gel electrophoresis. 2. Use freshly prepared RNA 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

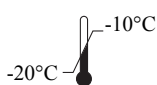
Hi-PCR® Peste des petits ruminants (PPR) Semi-Q PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal

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.



Storage temperature



Do not use if package is damaged



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Disclaimer :

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