

MBPCR212

Hi-PCR® Trypanosoma evansi Semi-Q PCR Kit

Description

Trypanosomes are unicellular flagellar protozoa belonging to the family of Trypanosomatidae and the genus *Trypanosoma*. The genus comprises of many species causing trypanosomoses disease in domestic and wild animals. One such species, *Trypanosoma evansi* causes an important disease called "Surra". The course of infection ranges from an acute disease with high mortality to a chronic infection characterized by subcutaneous edema, fever, lethargy, weight loss, abortion, nasal and ocular bleeding, and stiffness of the limbs. Surra can lead to neuropathy and immune suppression coupled with anemia eventually leading to death in both domestic and wild mammals. Clinical signs of neurological disorders are reported in horses, camels, buffaloes, cattle, deer and cats infected by *T. evansi*. Due to the inherent limitations of microscopy and serology based assays, parasitologists now use molecular methods such as target gene amplification. In recent years, several types of PCR assays have been used for diagnosis of trypanosomiasis in various parts of the world. PCR is considered the most accurate method for diagnosis of *T. evansi*.

NOTE: HiMedia's Hi-PCR® Trypanosoma evansi Semi-Q PCR Kit is for *in-vitro* use only.

Intended Use:

The Hi-PCR® Trypanosoma evansi Semi-Q PCR Kit is a qualitative conventional PCR kit which results in amplification of *T. evansi* DNA using specific primers. Conventional PCR testing can provide rapid, sensitive and specific detection of *T. evansi*.

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. HiMedia's Hi-PCR® Trypanosoma evansi Semi-Q PCR Kit is a qualitative conventional PCR kit which includes specific primers for the amplification of a 90 bp DNA fragment specific to *Trypanosoma evansi*. The amplified target is detected by using agarose gel electrophoresis based on separation of DNA fragments according to their size. This kit also contains a **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. This diagnostic kit assures sensitive detection in clinical samples. The kit is designed for *in-vitro* diagnostics and provides qualitative detection.

Features

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

Sample Source: Blood, tissue samples

Storage:

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. HiMedia does not recommend using the kit after the expiry date stated on pack.

Kit Contents:

The provided kit contains:

Components	Product codes	Reagents provided for (reactions)* (µL)	
		25R	50R
2X PCR TaqMixture	MBT061	675	1350
Primer Mix for <i>T. evansi</i>	DS0891	108	216
Positive Control for <i>T. evansi</i>	DS0894	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 50µl 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 in individual safety data sheets.

Sample Preparation:

Various clinical samples are routinely examined. For extraction and purification of pure bacterial DNA for high yield, perform the nucleic acid purification using HiMedia's HiPurA® Multi-Sample DNA Purification Kit (MB554) 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, 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	Product code	Recommended volume to be added per reaction (μL)
2X PCR TaqMixture	MBT061	25 μL
Primer Mix for <i>T. evansi</i>	DS0891	4 μL
Template (Extracted DNA) / Positive control	DS0894	5 μL
Molecular Biology Grade Water for PCR	ML065	Up to 50 μL

NOTE: For a Positive Control reaction tube, 5μL of positive control DNA (DS0894) is to be added along with above mentioned components instead of template (extracted DNA).

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). Interpret the data using Agarose Gel Electrophoresis.

B. Recommended PCR program:

1 Initial denaturation	: 95°C for 10 minutes	No. of cycles: 1
2 Denaturation	: 95°C for 45 seconds	
3 Annealing	: 55°C for 30seconds	No. of cycles: 30
4 Extension	: 72°C for 30 seconds	
5 Final Extension	: 72°C for 05 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.

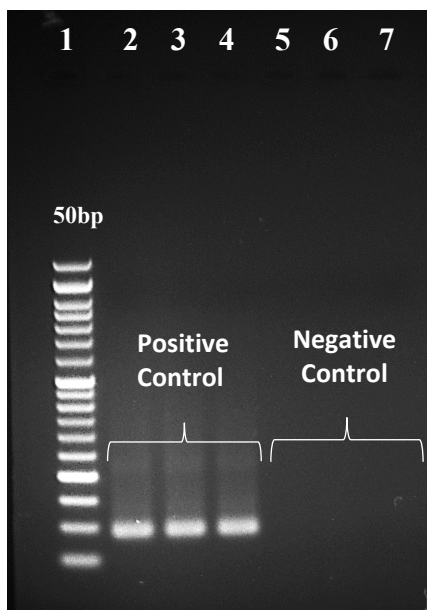
E. EtBr-staining to check results:

- 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.

F. Quality Control

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

G. Amplification Data:



Lane	Samples
1	50 bp DNA ladder
2, 3, 4	Positive control (90 bp)
5, 6, 7	Negative controls

Gel image representing amplification of *T. evansi* specific 90-bp target DNA fragment

Precaution

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 HiMedia's Hi-PCR® *Trypanosoma evansi* 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 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

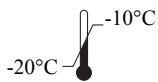
HiMedia's Hi-PCR® Trypanosoma evansi Semi-Q PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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 to mb@himedialabs.com.



Storage temperature



Do not use if package is damaged



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