# MBPCR214 Hi-PCR® Trypanosoma evansi SYBr PCR Kit

## Description

Trypanosomes are unicellular flagellar protozoa belonging to the family of Trypanosomatidae and the genus *Trypanosoma*. The genus *Trypanosoma* comprises many species causing diseases called trypanosomoses in domestic and wild animals. *Trypanosoma evansi* belongs to the subgenus Trypanozoon, which causes an important disease in animals known as "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 inherent limitations of microscopy and serology based assays parasitologists were influenced towards the use of gene amplification methods. 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 SYBr PCR Kit is for *in-vitro* use only.**

## Intended Use:

Recommended for sensitive and specific detection of Trypanosoma evansi in clinical samples.

## Principle

HiMedia's Hi-PCR® Trypanosoma evansi SYBr PCR Kit is designed for detection of internal transcribed spacer 1 (ITS) gene giving amplification of 90 bp product of *T. evansi*. This kit allowas rapid, sensitive and specific detection of *T.evansi.* 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.

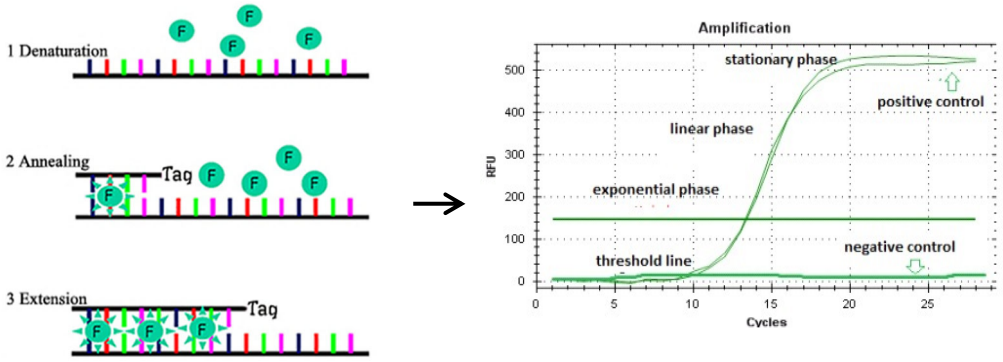
Real-time Polymerase Chain Reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of Polymerase Chain Reaction. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. Real-time PCR systems based on SYBr Green assays have increasingly been used for accurate, reliable detection and quantitation of various clinical pathogens. HiMedia's Hi-PCR® Trypanosoma evansi SYBr PCR Kit, is one such SYBr green based qPCR technique which allows amplification ITS – 1 gene.



ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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
Email : info@himedialabs.com
mb@himedialabs.com

**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
- Rapid detection of all relevant clinical pathogens

**Sample Source:** Blood, tissue 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 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 | 675 |
| Primer Mix for *T. evansi* | DS0892 | 27 | 54 |
| Positive Control for *T. evansi* | DS0894 | 25 | 50 |
| Molecular Biology Grade water for PCR | ML065 | 200 | 400 |

***** For a 25µl 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® Multi-Sample DNA Purification Kit (MB554)

**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 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 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) |
|---|---|---|
| Hi-SYBr master mix (with Taq Polymerase) | MBT074 | 12.5 |
| Primer Mix for *T. evansi* | DS0892 | 1.0 |
| Template DNA (Extracted DNA)/Positive Control for *T. evansi* | DS0894 | 5 |
| Molecular Biology Grade water for PCR | ML065 | Up to 25 |

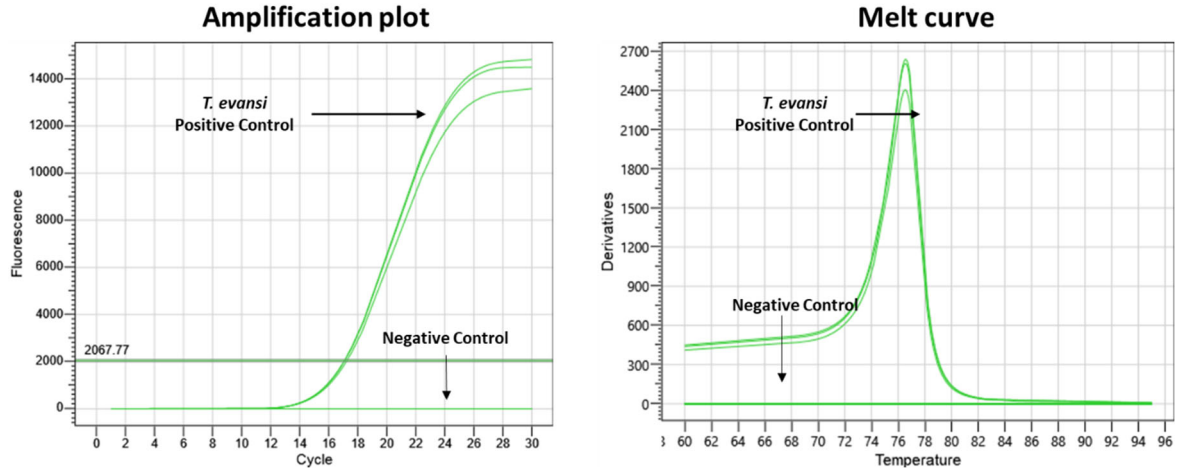
Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

**B. Recommended PCR program**

1. Initial denaturation : 95°C for 10 minutes — No. of cycles: 1
2. Denaturation : 95°C for 15 seconds
3. Annealing (Plate Read) : 60°C for 01 minute — No. of cycles: 30
4. 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.**

**Amplification data**




| Sr. No. | Sample | $C_t$ value | Tm Value |
|---|---|---|---|
| 1 | Positive control | 17.34 | 76.6 |
| 2 | Negative control | N/A | No Tm |

**Data representing real-time amplification of *T. evansi* Positive Control with $C_t$ values (provided in table)**

**Data interpretation**

| Melting Temperature (Tm)* | Result Interpretation |
|---|---|
| 74°C - 78°C | Positive for T. evansi |

**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 HiMedia's Hi-PCR® Trypanosoma evansi SYBr PCR Kit is tested against predetermined specifications to ensure consistent product quality.

**Quality Control**

Each lot of HiMedia's Hi-PCR® Trypanosoma evansi 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 | 1. Check the integrity of DNA using agarose gel electrophoresis.<br>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.<br>2. Repeat the analysis of all tests with fresh aliquots of critical reagents. |

**Safety Information**

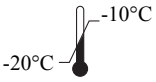
HiMedia's Hi-PCR® Trypanosoma evansi SYBr PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

**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 at 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


01/2027

PIMBPCR214_O/0124 MBPCR214-05