MBPCR224 Hi-PCR® Bluetongue virus (BTV) SYBr PCR Kit

Description

Bluetongue virus (BTV) belongs to family Retroviridae, genus *Orbivirus,* has segmented double stranded RNA genome is the causative agent of Bluetongue disease. BTV is transmitted to domestic and wild ruminants via few species of Culicoides spp. (i.e. biting midges). BTV can infect any ruminant species, including cattle, sheep, deer, goats and camelids, although severe clinical signs are most commonly seen in improved breeds of sheep. The severity of disease varies among different species with symptoms being most severe in sheep resulting in deaths, weight loss and disruption in wool growth. The majority of infected animals develop no detectable clinical signs but in others, signs may include fever, depression, lameness, oedema of the lips, tongue and head, conjunctivitis, coronitis, excessive salivation, nasal discharge, hyperaemia and pain at muco-cutaneous junctions such as the gums and vulva, and death. In pregnant animals abortion may also occur. The blue tongue after which the disease was named is seen only rarely and in more serious clinical cases. Following recovery, animals may also exhibit a number of long-lasting secondary effects, such as reductions in milk yield and weight gain, severe wool break and temporary infertility. Replacing the time consuming conventional diagnostic methods, molecular biological methods such as PCR have made a valuable contribution by greatly reducing diagnostic times while raising the reliability.

NOTE: Hi-PCR® Bluetongue virus (BTV) SYBr PCR Kit is for *in-vitro* use only.

Intended Use:

Recommended for sensitive and specific detection of Bluetongue virus in clinical samples.

Principle

Hi-PCR® Bluetongue virus (BTV) SYBr PCR Kit is designed for detection of specific sequence of the non-structural protein (NS) gene giving amplification of 82 bp product of Bluetongue virus 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® Bluetongue virus (BTV) SYBr PCR Kit is a qualitative Real-Time PCR kit which includes primers specific for the amplification of the non-structural protein gene (82 bp) of Bluetongue virus.

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

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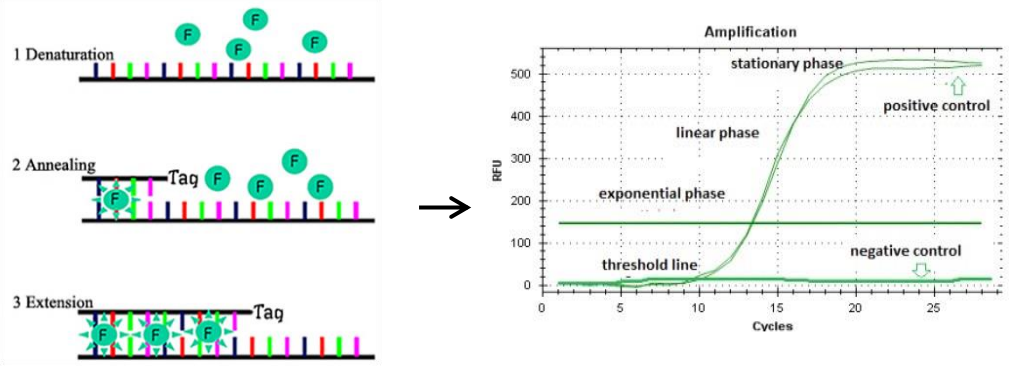
Diagrammatic representation of SYBr Green Chemistry in Real-Time PCR



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: Blood samples

Storage and Shelf-life

The provided kit has a shelf-life of 12 months when stored at -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
M-MuLV Reverse Transcriptase	DS0220	27	54
Primer Mix for BTV	DS0945	27	54
Positive Control for Bluetongue virus	DS1188	25	50
Molecular Biology Grade water for PCR	ML065	180	360

***For a 25 µL PCR reaction**

Materials needed but not provided:

All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q96®AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96®AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012

Insta Q48® M4 Real time PCR System, 96 well block, 4 channels	LA1023
TabSpin™ Microcentrifuge	LA1089/LA1090
Automated nucleic acid extraction system and materials	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag16, Insta NX® Mag16^{Plus}	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32^{Plus}	LA1096, MBLA019
Insta NX® Mag96	LA1097
Extraction Kits	
HiPurA® DNA/ RNA Purification Kit	MB583
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Cartridges)	MB583PC16200
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Plates)	MB583MPF16200
Tubes, plates and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615
µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18

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.

Protocol for PCR Reaction Mix Preparation

1. In the "Master mix Preparation" area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for 5 seconds. Keep on ice for later use.
2. Based on the total number of specimens (including PC and NTC) to be tested (N), calculate the volume of the components to be added as **N X volume of "1R"**
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix. Refer the following table. After all the reagents are added, mix them thoroughly and centrifuge for 5 seconds.

Components	Product code	Volume for "1R" (one reaction)
Preparation of PCR Reaction Mix		
Hi-SYBr master mix (with Taq Polymerase)	MBT074	12.5 µL
M-MuLV Reverse Transcriptase	DS0220	1.0 µL
Primer Mix for BTV	DS0945	1.0 µL
Molecular Biology Grade Water for PCR	ML065	5.5 µL
Total PCR Reaction Mix	**-**	**20.0 µL**
Template addition		
Template (Extracted RNA)		5.0 µL
Total reaction volume	**-**	**25.0 µL**

4. Aliquot **20µL of PCR reaction mix** into respectively labeled 0.1/0.2mL PCR tube/plate/strips, compatible to the PCR instrument to be used.
5. In the "Nucleic acid handling" area, add **5µL of extracted nucleic acid** of test specimen into the plate/strip to respective wells.
6. For positive and no template control, template nucleic acid is replaced by Positive control and nuclease free water, respectively. Refer the following table.

Set up of **Positive controls (PC)** for the PCR run		
Components	**Product code**	**Volume for "1R" (one reaction)**
Total PCR Reaction Mix	**-**	20.0 µL
Positive Control for Bluetongue virus	DS1188	5.0 µL
Total reaction volume	**-**	**25.0 µL**

Set up of **Negative Template controls (NTC)** for the PCR run		
Components	**Product code**	**Volume for "1R" (one reaction)**
Total PCR Reaction Mix	-	20.0 µL
Molecular Biology Grade water for PCR	ML065	5.0 µL
Total reaction volume	**-**	**25.0 µL**

7. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
8. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
9. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below).

Interpret the data from the amplification plot (observe the Ct values).

Recommended PCR program

1. Reverse transcription : 50°C for 15 minutes — No. of cycles: 1
   95°C for 05 minutes — No. of cycles: 1
2. Initial denaturation : 94°C for 15 seconds
3. Annealing (Plate Read) : 60°C for 20 seconds — No. of cycles: 40
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

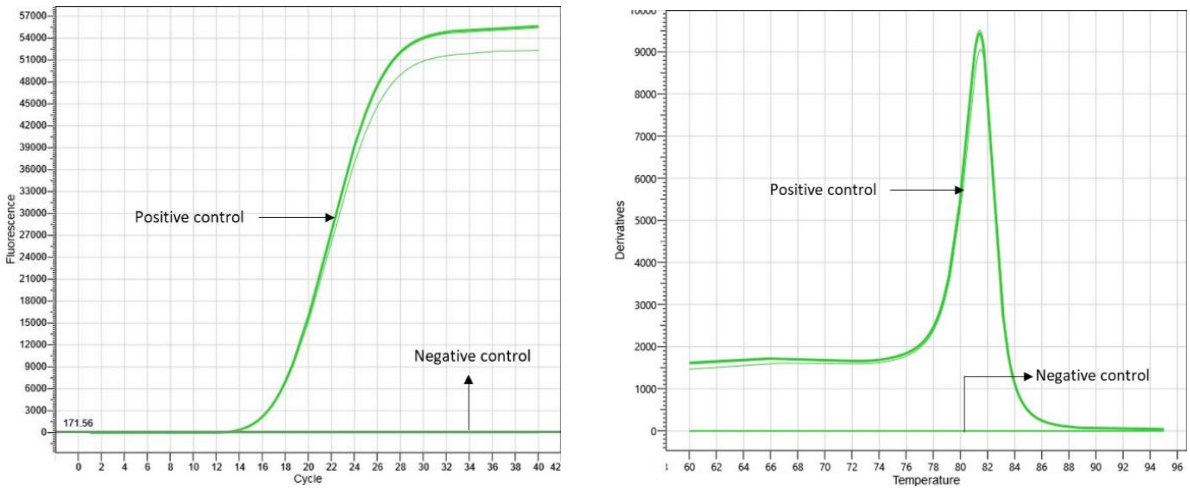
NOTE: The user can also set up a melt curve program as per their existing PCR instrument.

Quality Control

Each lot of HiMedia's Animal Bluetongue virus Detection Kit (Real-Time SYBr Based PCR) is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested for amplification.

A. Amplification data

Amplification plot **Melt curve**



Sr. No.	Sample	C_t value	Tm (°C)
1	Positive control	13.42	81.43
2	Negative control	N/A	No Tm

Image representing real-time amplification of Bluetongue virus (Ct and Tm values provided in table)

Data Interpretation

Melting Temperature (Tm)*	Result Interpretation
79°C-83°C	Positive for Bluetongue virus

*** Tm values can slightly vary for different sample types. If the Tm values show significant variation from those mentioned in the above table, then the sample is considered to be negative for Bluetongue virus.**

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® Bluetongue virus (BTV) SYBr PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® Bluetongue virus (BTV) 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. 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

Hi-PCR® Bluetongue virus (BTV) 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.

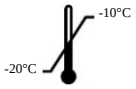
Disposal

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.

Symbols:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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