MBPCR185 Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR)

Description

Buffalos are considered as sacred animals that are part of rural livelihood and economic necessity in India. Therefore, to prevent illegal exploitation, a quick, sensitive and specific assay for detection of buffalo is important. In such cases, molecular genetic approaches are preferred because of their higher sensitivity and specificity, as well as rapid processing time and low cost as compared to other sensitive techniques. Nucleic acid amplification-based assays or Polymerase Chain Reaction (PCR) is an alternative method that allows for sensitive and specific detection of cytochrome b (cyb) region from blood / meat / tissue samples. Real-Time PCR technique is considerably simple and fast with respect to the standard PCR technique. This technique has been successfully used for the rapid detection and identification of a variety of infectious and non-infectious pathogens and genes.

NOTE: HiMedia's Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) is for forensic use only.

Intended Use

Recommended for sensitive and specific detection of Buffalo species. Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) is manufactured in accordance with ISO 18385:2016 guidelines.

Principle

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 PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) is designed to detect the **cytochrome b (cyb) region of Buffalo in FAM channel** with **Internal Control in JOE channel** in a single tube reaction. The kit allows sensitive and specific detection of Buffalo in a single tube reaction.

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.

Negative Control

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. It is recommended to have minimum 1 reaction of negative control per run.

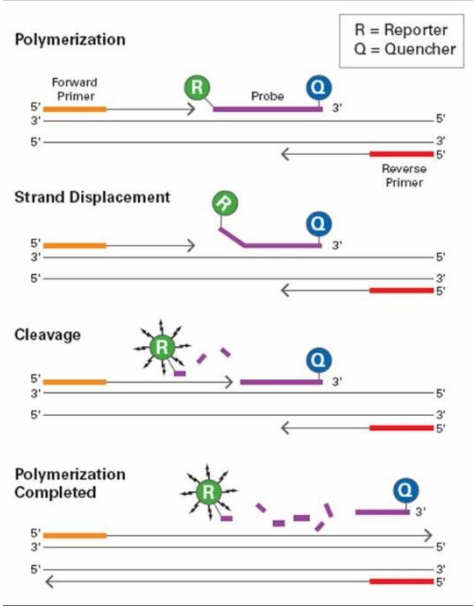
Internal Control

This is a control sequence which is amplified in the same reaction tube along with the target sequence (target species) but detected with a different primer (i.e. Multiplex PCR). Internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

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 preferential binding of probe specific to DNA fragments in Real-time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement: When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle,the DNA polymerae cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'- nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Molecular Features

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

Technology features:

- Fast and reliable results within 60 minutes.
- Includes all reagents & controls for validity of the test.
- Open system – Compatible with all qPCR cyclers.
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

Sample Source: Meat / Tissue / Blood 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 the 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 Laboratories does not recommend using the kit after the expiry date stated on pack.

Specimen 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 other body fluids. Safety guidelines may be referred to in individual safety data sheets.

Kit Contents:

The provided PCR kit contains:

Components	Product code	Reagents provided for * (µL)		
		25R	50R	100R
Buffalo PCR Master Mix	DS2791	338 µL	675 µL	1325 µL
Buffalo Primer-Probe Mix	DS0427	27 µL	54 µL	106 µL
Internal Control Primer-Probe Mix	DS1117	27 µL	54 µL	106 µL
Internal Control DNA	DS0385	27 µL	54 µL	106 µL
Buffalo Positive Control	DS0941	25 µL	50 µL	100 µL
Molecular Biology Grade Water for PCR	ML065	257 µL	513 µL	1007 µL

***** For a 25 µL PCR reaction

Materials needed but not provided

Appropriate real-time PCR instrument
Appropriate nucleic acid extraction system or kit
Centrifuge with a rotor for 1.5 mL - 2 mL reaction tubes
Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
Vortex mixer
PCR tubes (0.1ml or 0.2ml) or 96 well reaction plates with corresponding (optical) closing material or lid
Pipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)
Pipette tips with filters (As per pipette capacity)
Powder-free gloves (disposable)

All these 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
Insta Q48® M2 Real time PCR System, 96 well block, 2 channels	LA1024
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® Bacterial Genomic DNA Purification Kit	MB505
HiPurA® Multi-Sample DNA Purification Kit	MB554
HiPurA® Pre-filled Plates for Bacterial DNA Purification	MB505MPF-32
HiPurA® Pre-filled Plates for Food DNA Purification	MB505MPF16
HiPurA® Pre-filled Plates for Bacterial DNA Purification	MB505MPF-96
HiPurA® Pre-filled Cartridges for Bacterial DNA Purification	MB505PC16
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

Kit compatibility with Real-Time PCR Systems

Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) contains fluorophores that are compatible to the following PCR systems. Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) however has been validated on Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

Real-Time PCR system	Company	Dye 1	Dye 2
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	HEX
BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch	Bio-Rad Laboratories, Inc.	FAM	JOE/HEX
QuantStudio™ 5	Applied Biosystems	FAM	JOE/HEX/VIC
ABI® Prism SDS 7500	Applied Biosystems	FAM	JOE/HEX/VIC
QIAquant 96 & 384 5plex	QIAGEN	FAM	JOE/HEX
Rotor-Gene®6000 & Q	QIAGEN	FAM	JOE/HEX
LightCycler® 96 / LightCycler® 480	Roche	FAM	JOE/HEX/VIC
qTOWER^3	Analytik Jena	FAM	JOE/HEX/VIC

Note: Ensure that the Real-Time PCR system is calibrated for dyes mentioned above and maintained according to the manufactureŕs instructions and recommendations.

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 sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

Protocol for PCR Master 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 number of specimens to be tested (N), calculate the volume of the components to be added as N* volume of “1X”
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix of Tube 1, Tube 2 and Tube 3. Refer the following table. After all the reagents are added, mix them thoroughly and centrifuge for 5 seconds.

Components	Product code	Volume to be added for 1R (for a 25 µL reaction)
Buffalo PCR Master Mix	DS2791	12.5 µL
Buffalo Primer-Probe Mix	DS0427	1 µL
Internal Control Primer-Probe Mix	DS1117	1 µL
Internal Control DNA	DS0385	1 µL
Molecular Biology Grade Water for PCR	ML065	4.5 µL
Template DNA/Negative Control/Positive Control	-	5 µL
Total volume	-	25 µL

Centrifuge the tube briefly at 6000 rpm for about 10 seconds. 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).

A. Recommended PCR program

1. Initial denaturation : 95°C for 10 minutes
2. Denaturation : 95°C for 15 seconds
3. Annealing & Extension : 60°C for 30 seconds (Sampling) — No. of cycles: 40

   Channels : FAM/JOE

Selection of channels

Target	Channels	Quencher
Buffalo	FAM	None
Internal Control	HEX/JOE/VIC	None

Please select 'Passive reference dye' as 'None' wherever applicable

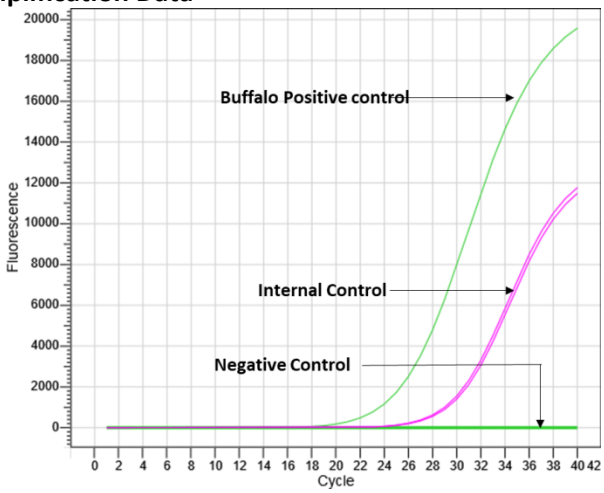
Data Analysis

The following conditions should be met for a valid diagnostic test:

Control	Detection channel	
	Buffalo (FAM)	Internal Control (JOE)
Positive Control	+	+
Negative Control	-	+

Ct value	Result
≤ 35	Detected (+)
> 35 or N/A	Not detected (-)

Amplification Data



Sr. No.	Sample	C_t value	
		PC	NC
1	Buffalo Positive control	25.48	-
2	Internal Control	30.0	29.5
PC: Positive Control, NC: Negative Control			

Image representing amplification plot of Buffalo DNA with Ct values using HiMedia's Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR). The results completely depend upon sample types.

Data Interpretation

Detection Channel		Result Interpretation
Buffalo (FAM)	Internal Control (JOE)	
+	+/-*	Positive for Buffalo
-	+	Negative for Buffalo
-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

*The presence or absence of a signal in the JOE channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

Analytical Performance

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the HiMedia's Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) was conducted on InstaQ96® Real Time PCR system and Bio-Rad CFX96™ C1000 Real Time PCR system. The detectable limit of the HiMedia's Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) on both instruments was determined to be 1 copies/reaction (**40 copies/mL**).

Inclusivity

In silico analysis for the assessment of inclusivity for the HiMedia's Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) was conducted by mapping the primers and probe against the available *Bubalus bubalis* sequences in GenBank. The HiMedia's Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) targets 100% of the known *Bubalus bubalis* strains.

Cross-reactivity - Analytical Specificity

In silico analysis was performed using NCBI nucleotide and Primer BLAST. The primers and probe for *Bubalus bubalis* specific cytochrome b (cyb) region were analyzed against organisms that are most frequently encountered in environments common for *Bubalus bubalis*.

Warning

Not for diagnosis. Not for Medicinal Use.

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 the guidelines established. Safety guidelines may be referred to in safety data sheets of the product.

Limitations

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in under quantity or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

Performance and Evaluation

Each lot of HiMedia's Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) has been functionally tested in amplification assay.

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 a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a real-time PCR instrument.
		Pipetting error	C_t values of replicates can show increased variation due to poor laboratory technique 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.
4.	No signal with positive controls	Incorrect programming of the temperature profile of the thermocycler	Compare the temperature profile to the manual.

Safety Information

HiMedia's Hi-PCR® Buffalo Detection Kit (Real-Time Probe Based PCR) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

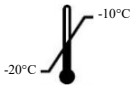
Disposal

Users 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 materials 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

Identification No.: PIMBPCR185
Rev.No.: 12
Date of Issue: 2026-01

HiMedia Laboratories Pvt. Ltd. Reg.office : 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 Email:mb@himedialabs.com Website: www.himedialabs.com