MBPCR141 Hi-PCR® Cow-Buffalo Probe PCR Kit

Description

The globalization of the food market and increased consumption of processed foods has increased chances of mixing declared food products by chance or with undeclared cheaper alternatives of food source. Even religious and ethical criteria can be at risk in the case of food adulteration. In order to verify the authenticity of products, food laboratories need reliable methods with sensitivity and specificity, which can answer these questions. Preventing adulteration of foodstuffs with less desirable or objectionable species is important for economic, regulatory health and cultural reasons. 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 milk 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® Cow-Buffalo Probe PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of Cow and / or Buffalo in food products.

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® Cow-Buffalo Probe PCR Kit is designed to detect the **cytochrome b (cyb) region** of **Cow and Buffalo in FAM and JOE channel,** respectively, with **Internal Control in Texas Red channel** 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

A Negative control is needed to ensure that the reagents, equipment, and environment used in the assay is 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). An 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

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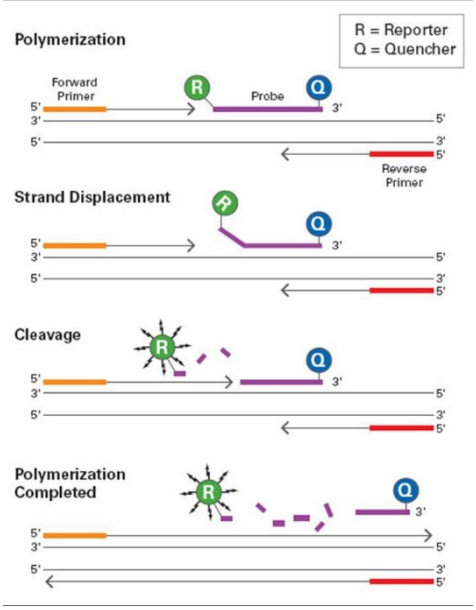
Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



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.

Features

- Fast and simple
- Good sensitivity and specific results
- Guaranteed reproducible results

Sample Source: Meat / Tissue / Blood / Milk and Food samples (raw and/or processed)

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.

Kit Contents: The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)	
		25R	50R
Hi-Quanti 2X Realtime PCR Master Mix	MBT180	338	675
Cow-Buffalo Primer-Probe Mix	DS1095	54	108
Internal Control Primer-Probe Mix	DS1097	27	54
Internal Control DNA	DS0385	27	54
Cow-Buffalo Positive Control	DS0939A	25	50
Molecular Biology Grade Water for PCR	ML065	200	400

* For a 25 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 in individual safety data sheets.

Sample Preparation

Various samples are routinely examined. For extraction and purification of pure DNA for high yield, perform the nucleic acid purification using HiMedia's extraction kits 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) & 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
- For Blood samples: HiPurA® Blood Genomic DNA Miniprep Purification Kit (MB504)
- For Meat / Tissue samples: HiPurA® Mammalian Genomic DNA Purification Kit (MB506)
- For Milk and Food samples (raw and/or processed): HiPurA® Food DNA Purification Kit (MB562)

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.

A. Protocol for PCR Master Mix Preparation

Components	Product Code	Volume to be added in µl for 1R (for a 25 µL reaction)
Hi-Quanti 2X Realtime PCR Master Mix	MBT180	12.5 µL
Cow-Buffalo Primer-Probe Mix	DS1095	2 µL
Internal Control Primer-Probe Mix	DS1097	1 µL
Internal Control DNA	DS0385	1 µL
Molecular Biology Grade Water for PCR	ML065	3.5 µL
Template DNA / Positive Control / Negative 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).

B. Recommended PCR program

1. Initial denaturation : 95°C for 10 minutes
2. Denaturation : 95°C for 15 seconds
3. Annealing : 60°C for 30 seconds (Sampling) — No. of cycles: 40
   Channels : FAM/JOE/Texas Red
4. Hold : 4°C for ∞

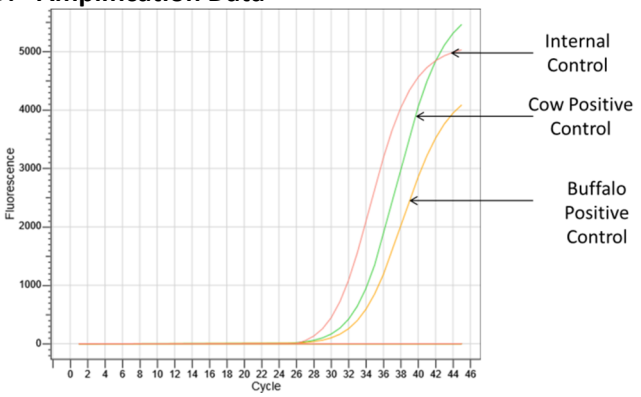
C. Data Analysis

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

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

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

D. Amplification Data



Sr. No.	Sample	C_t value	
		PC	NC
1	Cow Positive Control	25.6	-
2	Buffalo Positive Control	26.7	
3	Internal Control	29.7	29.5
PC: Positive Control, NC: Negative Control			

Image representing amplification plot of Cow and Buffalo DNA with Ct values using HiMedia's Hi-PCR® Cow-Buffalo Probe PCR Kit. The results completely depend upon sample types.

E. Data Interpretation

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

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

Calculation of Cow-Buffalo Percentage:

Cow-Buffalo % can be calculated with the help of the following formula: **Cow Ct - Buffalo Ct**

Cow Milk (%)	Buffalo Milk (%)	Ct Difference
100	0	NA
> 90	< 10	≥ -3.51
90	10	-3.5 to -2.51
80	20	-2.5 to -1.51
70	30	-1.5 to -0.51
60	40	-0.5 to 0.51
50	50	0.5 to 1.49
40	60	1.5 to 2.49
30	70	2.5 to 3.49
20	80	3.5 to 4.49
10	90	4.5 to 5.49

< 10	> 90	≥ 5.5
0	100	NA

NA: Not applicable

Analytical Performance

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the HiMedia's Hi-PCR® Cow-Buffalo Probe PCR Kit was conducted using clinical specimens on InstaQ96® Real Time PCR system and Bio-Rad CFX96™ C1000 Real Time PCR system. The detectable limit of the HiMedia's Hi-PCR® Cow-Buffalo Probe PCR Kit 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® Cow-Buffalo Probe PCR Kit was conducted by mapping the primers and probe against the available *Bos taurus* sequences in GenBank. The HiMedia's Hi-PCR® Cow-Buffalo Probe PCR Kit targets 100% of the known *Bos taurus* strains.

Cross-reactivity - Analytical Specificity

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

Warning

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 established guidelines. Safety guidelines may be referred 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 quantitation 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® Cow-Buffalo Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Cow-Buffalo Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA 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 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

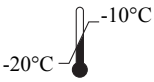
HiMedia's Hi-PCR® Cow-Buffalo Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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

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