

## **MBPCR186      Hi-PCR® Cattle Sex Determination Kit (Real-Time Probe Based PCR)**

### **Description**

Determination of sex origin of cattle meat has been always of public interest in countries such as India, where the slaughter of cows (female cattle) is banned because of religious beliefs and laws. To implement the regulations pertaining to such issues there is need to have reliable, authentic, and simple methods for determining the sex of cattle. Over the last few decades, DNA-based techniques, especially polymerase chain reaction (PCR)-based methods for meat sex determination, have received particular attention and have proved to be reliable, sensitive, and rapid. DNA regions that differ between male and female individuals are essential features in PCR sex determination. More recently, based on real time PCR, probe technology for bovine sex determination is reliable. Nucleic acid amplification-based assays or Polymerase Chain Reaction (PCR) is an alternative method that allows for sensitive and specific detection of the X chromosome and the Y chromosome from blood / meat / tissue. 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® Cattle Sex Determination Kit (Real-Time Probe Based PCR) is for *in-vitro* use only.

### **Intended Use**

Recommended for sensitive and specific detection of Cattle Sex.

### **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® Cattle Sex Determination Kit (Real-Time Probe Based PCR) is designed to detect the X chromosome in FAM channel and the Y chromosome in JOE channel 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.

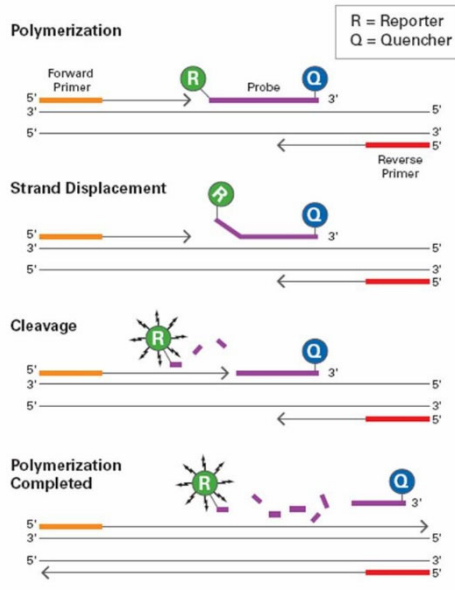
### **Negative Control**

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.

## 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 polymerase 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 samples

### Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -15°C to -25°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 * (µL)
		100R
4X Super Mastermix	DS0900A	663
X-chromosome Primer-probe mix	DS0453	106
Y-chromosome Primer-probe mix	DS0890	106
Internal Control Primer-Probe Mix	DS1097	106
Internal Control DNA	DS0385	106
XY Positive Control	DS0457	100
Molecular Biology Grade Water for PCR	ML065	800

\* 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/LA1073/LA1023/LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes
- For blood samples / meat / tissue samples: HiPurA® Forensic Multi Sample DNA Purification Kit (MB580)

### 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 for 1R (for a 25 µL reaction)
4X Super Mastermix	DS0900A	6.25 µL
X-chromosome Primer-probe mix	DS0453	1 µL
Y-chromosome primer-probe mix	DS0890	1 µL
Internal Control Primer-Probe Mix	DS1097	1 µL
Internal Control DNA	DS0385	1 µL
Molecular Biology Grade Water for PCR	ML065	9.75 µL
Template DNA/Negative Control/Positive Control	DS0457	5 µL
Total volume		25 µL

**Note: The template volume can be increased by adjusting the volume of Molecular Biology Grade Water for PCR.**

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. UNG Incubation                    | : 25°C for 2 minutes                                    | } No. of cycles: 40 |
| 2. Initial denaturation              | : 95°C for 2 minutes                                    |                     |
| 3. Denaturation                      | : 95°C for 10 seconds                                   |                     |
| 4. Annealing & Extension<br>Channels | : 60°C for 30 seconds (Sampling)<br>: FAM/JOE/Texas Red |                     |
| 5. Hold                              | : 4°C for ∞   |                     |

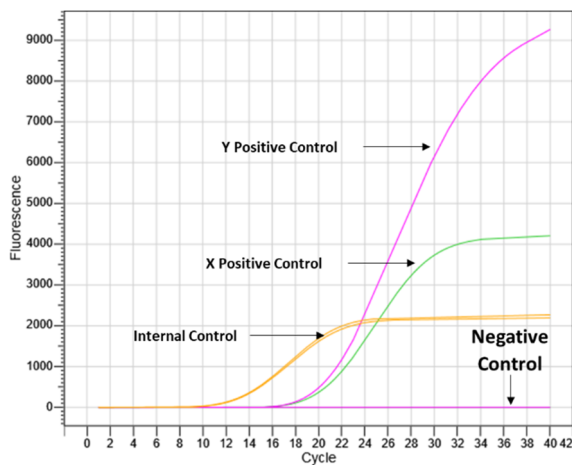
### C. Data Analysis

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

Control	Detection channel		
	FAM (X-Chromosome)	JOE (Y-Chromosome)	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 <sub>t</sub> value	
		PC	NC
1	X chromosome positive control	20.76	-
2	Y chromosome positive control	21.06	-
3	Internal Control	13.72	13.70

PC: Positive Control, NC: Negative Control

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

### E. Data Interpretation

Detection Channel			Result Interpretation
FAM (X-Chromosome)	JOE (Y-Chromosome)	Texas Red (Internal Control)	
+	-	+/-*	Positive for Female
+	+	+/-*	Positive for Male
-	-	+	Negative for both Male and Female
-	-	-	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.

### Analytical Performance

#### Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® Cattle Sex Determination Kit (Real-Time Probe Based PCR) was conducted on InstaQ96® Real Time PCR system and Quant Studio™ 5 Real-Time PCR system. The detectable limit of the HiMedia's Hi-PCR® Cattle Sex Determination Kit (Real-Time Probe Based PCR) on both instruments was determined to be **3 copies/ μL**.

**Inclusivity**

*In-silico* analysis for the assessment of inclusivity for the Hi-PCR® Cattle Sex Determination Kit (Real-Time Probe Based PCR) was conducted by mapping the primers and probe against the available sequences in GenBank. Hi-PCR® Cattle Sex Determination Kit (Real-Time Probe Based PCR) targets 100% of the known bovine strains.

**Cross-reactivity - Analytical Specificity**

*In-silico* analysis was performed using NCBI nucleotide and Primer BLAST. The primers and probe for X chromosome and Y chromosome were analyzed against other animal species and determined to be 100% specific.

**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® Cattle Sex Determination 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® Cattle Sex Determination Kit (Real-Time Probe Based PCR) 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 <sub>t</sub> 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

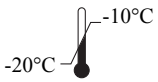
HiMedia's Hi-PCR® Cattle Sex Determination Kit (Real-Time Probe Based PCR) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

### Disposal

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](mailto:mb@himedialabs.com).



Storage temperature



Do not use if package is damaged



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**Disclaimer :**

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