MBPCR113 Hi-PCR® Brucella Semi-Q PCR Kit

Description

Brucellosis is an infectious disease caused by bacteria in the genus *Brucella* which is aerobic, gram-negative coccobacilli. Brucellosis is a zoonotic infection (meaning the disease occurs mainly in animals but is occasionally transferred to humans). General symptoms of brucellosis are often vague and similar to the flu. They may include- Fever, body-wide aches and pains, poor appetite and weight loss. Aborted fetuses, placental membranes or fluids, and other vaginal discharges present after an infected animal has aborted or calved are all highly contaminated with infectious *Brucella* organisms. The disease may also be spread when wild animals or animals from an affected herd mingle with brucellosis-free herds. The kit is designed to detect specific DNA sequence of *Brucella* gene. PCR testing can provide rapid, sensitive and specific detection of *Brucella*.

NOTE: HiMedia's Hi-PCR® Brucella Semi-Q PCR Kit is for *in-vitro* use only.

Intended Use:

The Hi-PCR® Brucella Semi-Q PCR Kit is a qualitative conventional PCR kit which results in amplification of *Brucella* specific gene using specific primers. Conventional PCR testing can provide rapid, sensitive and specific detection of *Brucella.*

Principle

HiMedia's Hi-PCR® Brucella Semi-Q PCR Kit is a qualitative conventional PCR kit which includes the amplification of (IS*711*) gene (113 bp) of *Brucella* using specific primers. The amplified target is detected by using agarose gel electrophoresis. Gel electrophoresis is used to analyze the amplification of desired gene region for target pathogen based on separation of DNA fragments according to their size. 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.

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.

Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of genes. The three steps of a successful PCR reaction include Denaturation, Annealing and Extension. The double-stranded DNA melts and forms single stranded DNA at high temperature (Denaturation). Sequence specific primers bind to the target sequence on single-stranded DNA at lower temperature (Annealing). *Taq* DNA Polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (Extension). These 3 steps of PCR are usually repeated between 30 to 40 times in each PCR assay.

Gel electrophoresis is used to analyze the amplification of desired gene region for target pathogen based on separation of DNA fragments according to their size.

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

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Features

- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results

Sample Source: Blood, tissue, fetal samples

Storage and Shelf-life

The provided kit has a shelf-life of 12 months when stored between -10° 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)*(µL)	
		25R	**50R**
2X PCR TaqMixture	MBT061	675	1350
Brucella Primer Mix	DS0541C	81	162
Brucella Positive Control	DS0904C	25	50
Molecular Biology Grade Water for PCR	ML065	500	1000
6X Gel Loading Buffer	ML015	54	108
50 bp DNA Ladder	MBT084	15	30

*For a 50 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 to individual safety data sheets.

Sample Preparation

Various clinical samples are routinely examined. For extraction and purification of pure bacterial DNA for high yield, perform the nucleic acid purification using HiMedia's HiPurA® Multi-Sample DNA Purification Kit (MB554) 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 Films (Product code: PR18)
- Thermal Cycler (Product Code: LA948 / LA949 / LA950 / LA1006 / LA1015/ LA1059 / LA1060 / LA1066)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes

General Preparation Instructions

- Before use, a suitable amount of all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- 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)
2X PCR TaqMixture	MBT061	25
Brucella spp. Primer Mix	DS0541C	3
Template (Extracted DNA) / *Brucella* species positive control	DS0904C	5
Molecular Biology Grade Water for PCR	ML065	Up to 50

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 using Agarose Gel Electrophoresis.

B. Recommended PCR program

1.	Initial denaturation	:	95°C for 10 minutes	No. of cycles: 1
2.	Denaturation	:	95°C for 30 seconds	No. of cycles: 30
3.	Annealing	:	60°C for 45 seconds	
4.	Extension	:	72°C for 30 seconds	
5.	Final Extension	:	72°C for 05 minutes	No. of cycles: 1

After amplification, the products may be kept at 4°C overnight or frozen at –20°C for long-term storage.

C. PCR Assay Results Interpretation

- For analysis of the PCR data, load 10 µL of amplicon on a 2% Agarose gel along with 1 µL of 6X Gel Loading Buffer (ML015).
- Load 3 µL of 50 bp DNA ladder (MBT084) in separate well.

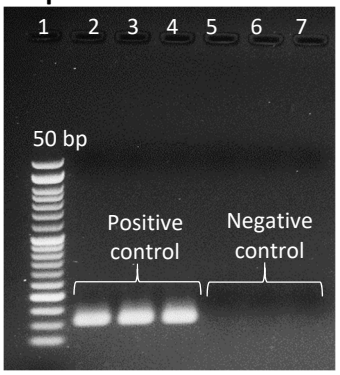
D. EtBr-staining staining to check results

- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 minutes.
- Confirm the expected amplicon size comparing with 50 bp DNA marker.

Quality Control

Each lot of HiMedia's Hi-PCR® Brucella Semi-Q PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific RNase / DNase activities. Functionally tested for amplification.

Amplification Data



Lane	Sample
1	50bp DNA Ladder
2,3,4	Amplicon of *Brucella* species (113 bp)
5,6,7	Negative Control

Gel Image representing amplification of IS711 gene for *Brucella* detection (113 bp)

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.

Performance and Evaluation

Each lot of HiMedia's Hi-PCR® Brucella Semi-Q PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
	No amplification	Degraded samples	Check the integrity of DNA using agarose gel electrophoresis.
			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.
	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.
	Amplification in negative control	Reagents contaminated	Replace all critical solutions. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

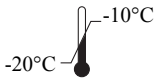
HiMedia's Hi-PCR® Brucella Semi-Q PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal

The 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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Disclaimer :

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