MBPCR121 Hi-PCR® Brucella SYBr 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* species gene. PCR testing can provide rapid, sensitive and specific detection of *Brucella* spp.

NOTE: Hi-PCR® Brucella SYBr PCR Kit is for *in-vitro* use only.

Intended Use:

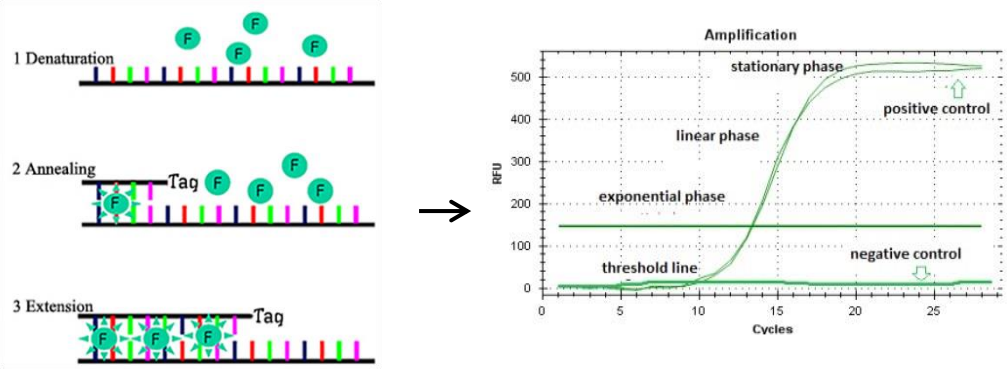
Recommended for sensitive and specific detection of *Brucella* species in clinical samples.

Principle

Hi-PCR® Brucella SYBr PCR Kit is designed for detection of specific sequence of multiple insertion element (IS*711*) gene giving amplification of 113 bp product of *Brucella* species in clinical samples. This kit also contains Positive control.

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® Brucella SYBr PCR Kit is a qualitative Real-Time PCR kit which includes the amplification of (IS*711*) gene (113 bp) of *Brucella* species.

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.

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

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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.

No Template Control
A No Template Control (NTC) is needed to ensure that the reagents, equipment, and environment used in the assay are not contaminated with target DNA/RNA. In this reaction, nuclease free water is used as the template. It is recommended to have a minimum of one reaction of no template control per run.

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

Sample Source: Blood, tissue samples

Storage and Shelf-life
The provided kit has a shelf-life of 12 months when stored between -10°C and -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
Brucella Hi-SYBr master mix	DS2310	338	675
Brucella Primer Mix	DS0541C	41	81
Brucella Positive Control	DS0904D	25	50
Molecular Biology Grade water for PCR	ML065	187	374

***For a 25 µL PCR reaction**

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
- HiPurA® Multi-Sample DNA Purification Kit (MB554)

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, 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)
Hi-SYBr master mix (with Taq Polymerase)	DS2310	12.5
Brucella Primer Mix	DS0541C	1.5
Molecular Biology Grade water for PCR	ML065	6.0
Template (Extracted DNA)/ Positive Control (DS0904D)/ NTC (Molecular Biology Grade water for PCR)	-	5.0
	Total volume	**25.0**

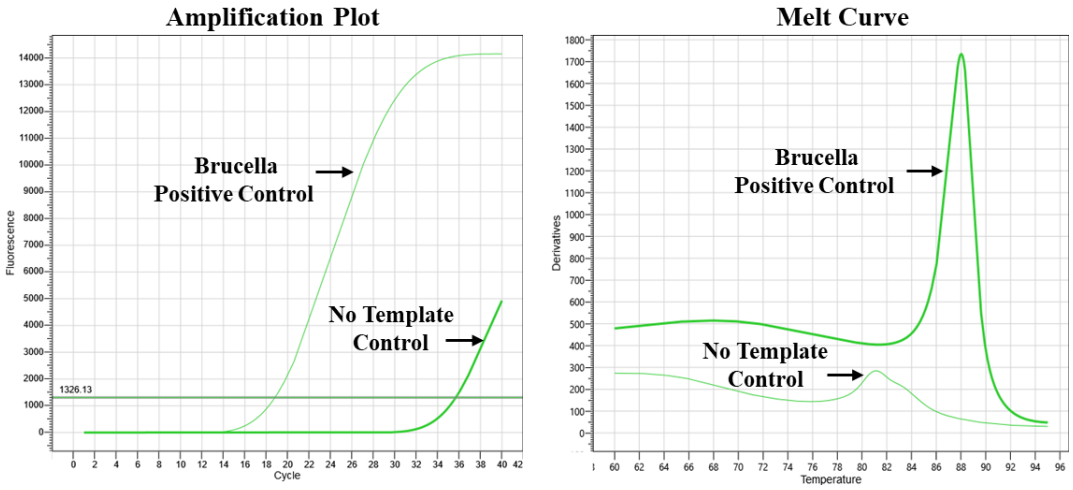
Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below).

B. Recommended PCR program

1. Initial denaturation : 94°C for 5 minutes — No. of cycles: 1
2. Denaturation : 94°C for 1 minute
3. Annealing (Plate Read) : 64°C for 1 minute — 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.

C. Amplification data



Sr. No.	Sample	C_t value	Tm Value
1	Brucella Positive control	18.99	88.03
2	No Template control	35.33	81.00

Figure: Data representing real-time amplification of *Brucella* species (provided in table)

Data Interpretation

Melting Temperature (Tm)*	Result Interpretation
86.5°C-89°C	Positive for *Brucella* species

*** 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 *Brucella*.**

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® Brucella SYBr PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® Brucella 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 No Template Control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

The Hi-PCR® Brucella 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.

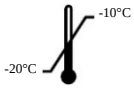
Disposal

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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