MBPCR117 Hi-PCR® Babesia bigemina Semi-Q PCR Kit

Description

Bovine babesiosis is a tick-borne disease of cattle caused by the protozoan parasites of the genus *Babesia* (Apicomplexa: Piroplasmorida) leading to significant economic losses in tropical and subtropical regions. More than 100 species of Babesia are recognized to infect domestic animals, wild animals, and humans worldwide. The principal species of *Babesia* that cause Bovine babesiosis are *Babesia bigemina*, *Babesia bovis* and *Babesia divergens*. *Babesia bigemina*, a major Babesia species responsible for bovine babesiosis is transmitted by *Rhipicephalus spp.* ticks in tropical and subtropical countries. Clinical manifestations of disease associated with *Bovine babesiosis* includes fever and intravascular hemolysis leading to progressive anemia, hemoglobinuria, and jaundice, and may result in death. The diagnostic methods of bovine babesiosis have relied mostly on clinical signs, microscopic examination of the parasites and serological testing for antibody detection. The advent of molecular diagnostic tests like polymerase chain reaction (PCR) has paved the way to efficient diagnosis than the conventional techniques. The Hi-PCR® Babesia bigemina Semi-Q PCR Kit is designed for specific and sensitive detection of *Babesia bigemina*.

NOTE: Hi-PCR® Babesia bigemina Semi-Q PCR Kit is for *in vitro* use only.

Intended Use

The Hi-PCR® Babesia bigemina Semi-Q PCR Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of PCR and in vitro diagnostic procedures. The kit is recommended for sensitive and specific detection of *Babesia bigemina* in clinical samples.

Principle

The Hi-PCR® Babesia bigemina Semi-Q PCR Kit is a qualitative conventional PCR kit for detection of *Babesia bigemina* in clinical samples. The PCR is performed for amplification of the 18S ribosomal RNA gene (165 bp) of *Babesia bigemina* using specific primers. The amplified target is confirmed using agarose gel electrophoresis. The presence of 165bp 18S ribosomal RNA gene product indicate *Babesia bigemina* infection. This kit also contains synthetic Positive control for validation of the test.

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.

Agarose gel electrophoresis is one of technique used to visualize and confirm the product/s formed in a PCR reaction. The amplification products/fragments are separated on gel according to their size.

Positive control

A Positive control (PC) is a control reaction which contain the target DNA sequence that the PCR is designed to amplify. It is used to ensure proper and intended functioning of all the PCR reagents and is recommended to be used in every run to assess optimal assay performance.

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

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Negative Control

A Negative Template Control (NTC) is essential to verify that the reagents, equipment, and environment used in the assay are free from contamination with target nucleic acid. In this control reaction, nuclease-free water is used as the template. It is recommended to include at least one negative template control reaction per run to ensure the reliability of the results.

Features

- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results
- Includes all reagents and controls

Sample source: Blood samples

Storage and Shelf-life

The 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
B. bigemina Semi-Q Master mix	DS2678	108	216
B. bigemina Primer Mix	DS0243	27	54
B. bigemina Semi-Q Positive Control	DS0272	75	150
6X Gel Loading Buffer	ML015	54	108
50 bp DNA Ladder	MBT084	15	30
Molecular Biology Grade Water for PCR	ML065	75	150

*For a 20 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.

Materials needed but not provided: All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Thermal Cycler	LA948 /LA949 / LA950 / LA1006 / LA1015/ LA1059 / LA1060 / LA1066
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® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Cartridges)	MB583PC16200
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Plates)	MB583MPF16200
HiPurA® DNA/ RNA Purification Kit	MB583
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

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.

Protocol for PCR Reaction 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 total number of specimens (including PC and NTC) to be tested (N), calculate the volume of the components to be added as **N X volume of "1R"**
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix. Refer the following table. After all the reagents are added, mix them thoroughly and centrifuge for 5 seconds.

Components	Product code	Volume for "1R" (one reaction)
Preparation of PCR Reaction Mix		
B. bigemina Semi-Q Master mix	DS2678	4.0 µL
B. bigemina Primer Mix	DS0243	1.0 µL
Total PCR Reaction Mix	-	**5.0 µL**
Template addition		
Template (Extracted DNA)		15.0 µL
Total reaction volume	**-**	**20.0 µL**

4. Aliquot **5 µL** of **PCR reaction mix** into 0.1/0.2mL PCR tube/plate/strips, compatible to the PCR instrument to be used.
5. In the "Nucleic acid handling" area, add **15 µL** of extracted nucleic acid of test specimen into the plate/strip to respective wells.

6. For positive and negative control, template nucleic acid is replaced by Positive control mix and nuclease free water respectively. Refer the following table.

Set up of **Positive controls (PC)** for the PCR run		
Components	**Product code**	**Volume for "1R" (one reaction)**
PCR Reaction Mix	-	5.0 µL
B. bigemina Semi-Q Positive Control	DS0272	15.0 µL
Total reaction volume	**-**	**20.0 µL**

Set up of **Negative Template controls (NTC)** for the PCR run		
Components	**Product code**	**Volume for "1R" (one reaction)**
PCR Reaction Mix	-	5.0 µL
Molecular Biology Grade water for PCR	ML065	15.0 µL
Total reaction volume	**-**	**20.0 µL**

7. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
8. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
9. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below).

Recommended PCR program

1. Initial denaturation	:	95°C for 05 minutes	No. of cycles: 1
2. Denaturation	:	95°C for 30 seconds	No. of cycles: 45
3. Annealing and Extension	:	67°C for 30 seconds	

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

Agarose gel electrophoresis of PCR products:

- 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 4 µL of 50 bp DNA ladder (MBT084) in separate well.
  (Note: EtBr or any other Nucleic acid staining dyes can be incorporated in the agarose gel or stained after the gel run as per the manufacturers instruction).
- Confirm the PCR amplicon size comparing with 50 bp DNA marker.
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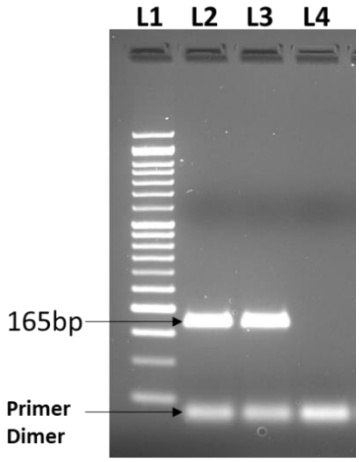
PCR Assay Results Interpretation



Lane	Sample
L1	50 bp DNA Ladder
L2, L3	*B. bigemina* Positive control (165 bp)
L4	Negative Template control

Gel Image representing amplification of 18s rRNA gene of *Babesia bigemina* (165bp)

A positive PCR result i.e. visible 165bp band in the sample indicates the presence of *B. bigemina* DNA in the sample, suggesting an active Babesiosis infection.

A negative PCR result i.e. absence of 165bp band in clinical sample suggests the absence of detectable *B. bigemina* DNA, but it doesn't definitively rule out Babesiosis infection, especially in early infection stages and if caused by other Babesia species. It may be crucial to consider other diagnostic methods such as serology and clinical findings.

Kindly correlate the results with clinical findings. Diagnosis generally relies on combination of clinical symptoms, PCR results, serological tests, etc.
Note: Data on the accuracy of specimen type recommended for diagnosis is limited for *Babesia bigemina* detection, therefore a negative sample specimen should be interpreted with caution.

Quality Control
Each lot of Hi-PCR® Babesia bigemina Semi-Q PCR Kit is functionally tested in amplification assays. The Hi-PCR® Babesia bigemina Semi-Q PCR Kit provides reagents for controls: *B. bigemina* Positive Control mix (PC) and a No Template Control (NTC) which are to be included in every run.

Warning and Precautions
Not for Medicinal Use. Read the procedure carefully before beginning the protocol. 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® Babesia bigemina Semi-Q PCR Kit is tested against predetermined specifications to ensure consistent product quality.

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	Check the PCR conditions in the machine and make appropriate changes wherever required
2.	Variability between replicates	Error in reaction set-up	1. Verify that the correct reagent volumes, dilutions and storage conditions have been used. 2. 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 negative control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

Hi-PCR® Babesia bigemina 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.

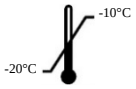
Disposal

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.

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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