MBPCR013 Hi-PCR® White Spot Syndrome Virus (WSSV) Semi-Q PCR Kit

Description

White Spot Syndrome Virus (WSSV) is a major shrimp disease, which has caused high mortality rate and economic losses to the major shrimp farming countries in South East Asia, Central America and Southern USA. It is a pathogen found in different penaeid shrimp species including P. monodon, *P. japonicus* and *L. vannamei* as well as other crustaceans, such as crab and crayfish. Molecular methods like PCR (Polymerase Chain Reaction) for pathogen detection have been developed for shrimp diseases. At present, PCR method is recognized to be the most effective diagnostic tool for this pathogen. In addition, a conserved segment of WSSV has also been selected as the target for PCR diagnosis.

NOTE: HiMedia's Hi-PCR® White Spot Syndrome Virus (WSSV) Semi-Q PCR Kit is for *in-vitro* use only.

Intended Use:

The Hi-PCR® White Spot Syndrome Virus (WSSV) Semi-Q PCR Kit is designed for detection of specific sequence of WSSV. Conventional PCR testing can provide rapid, sensitive and specific detection of WSSV.

Principle

HiMedia's Hi-PCR® White Spot Syndrome Virus (WSSV) Semi-Q PCR Kit is a qualitative conventional PCR kit which includes amplification using specific primers. The amplified target is confirmed by using agarose gel electrophoresis. The Hi-PCR® White Spot Syndrome Virus (WSSV) Semi-Q PCR Kit is designed to detect the specific gene regions of 146 F2/146 R2, an insertion element found exclusively within the members of the Shrimp samples. This kit contains an Internal and Positive control.

Internal control

This is a control sequence, which is amplified in the same reaction tube along with the target sequence (target pathogen) 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.

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.

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 a 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 25 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
- Rapid detection of all relevant clinical pathogens

Sample Source: Shrimp samples

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 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
Primer Mix for WSSV	DS0744	54	108
Primer Mix for Internal Control (285 bp)	DS0223	54	108
Molecular Biology Grade Water for PCR	ML065	500	980
6X Gel Loading Buffer	ML015	54	108
100 bp DNA Ladder	MBT049	20	40
Positive Control (WSSV DNA)	DS0318	10	20
Internal Control DNA	DS0123	27	54

***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:

For preparation of Shrimp DNA perform the nucleic acid purification using HiMedia's HiPurA® Shrimp DNA Purification Kit (MB581) as described 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	Recommended volume to be added per reaction (µL)
2X PCR TaqMixture	25
Primer Mix for WSSV	2
Primer Mix for Internal Control (285 bp)	2
Template	5
Internal Control DNA	1
Molecular Biology Grade Water for PCR	Up to 50

NOTE: (Optional) – The user can also set up an additional PCR reaction containing 2 µL of Positive control DNA (provided) in a separate tube.

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	:	94°C for 5 minutes	No. of cycles: 1
2.	Denaturation	:	94°C for 30 seconds	No. of cycles: 30
3.	Annealing	:	55°C for 30 seconds	
4.	Extension	:	72°C for 30 seconds	
5.	Final Extension	:	72°C for 3 minutes	No. of cycles: 1

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

D. PCR Assay Results Interpretation

- For analysis of the PCR data, load 10 µL of amplicon on a 1.5% Agarose gel along with 1 µL of 6X Gel Loading Buffer (ML015).
- Load 4 µL of 100 bp DNA ladder (MBT049) in separate well.

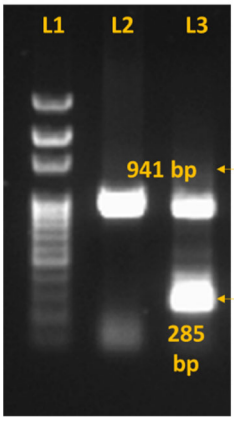
E. EtBr-staining staining to check results

- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 minutes.
- Confirm the expected amplicon size comparing with 100 bp DNA marker.
- Alternatively, the PCR products can be loaded in Pre-Poured™ Gel-665-12-2.

Quality Control

Each lot of HiMedia's Hi-PCR® White Spot Syndrome Virus (WSSV) Semi-Q PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific RNase / DNase activities. Functionally tested for amplification.

Amplification Data



Lane no.	Samples
1	100 bp ladder
2	Amplicon of WSSV DNA (941bp)
3	Amplicon of WSSV DNA (941bp) with Internal Control (285bp)

Gel image representing amplification of inner primer using target sample of WSSV sample with positive control (941 bp) and internal control (285 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® White Spot Syndrome Virus (WSSV) 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	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 negative control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

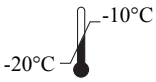
HiMedia's Hi-PCR® White Spot Syndrome Virus (WSSV) Semi-Q PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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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