White Spot Syndrome Virus (WSSV)

Catalog Number: NA2023

For Research Use Only.
Not for use in Diagnostic Procedures.

1. Description

WSSV is a double stranded DNA virus which causes a highly lethal disease (white spot syndrome) in many shrimps. As the name suggests, infected shrimps harbor white spots (0.5 to 2.0 mm in diameter) on the inner surface of their appendages. These shrimps also display red discoloration and have loose cuticles. As this disease is highly contagious and can wipe out many populations of many shrimp farms, early detection of the virus is imperative.

2. Features

This kit comes with a 2X qPCR Master Mix (UDG) which is a ready-to-use reagent for Real-Time PCR assays, containing:

- Hot Start-Taq DNA Polymerase
- PCR buffer
- MgCl2
- dATP, dCTP, dGTP, dUTP
- Uracil DNA Glycosylase (UDG)

- UDG prevents carry-over contamination by removing uracil (U) from single- or double-stranded DNA, preventing dU-containing DNA from being used as a template for subsequent PCR amplification.
- This kit also contains an internal control that detects DNA sequence of decapods including shrimp.

3. Storage and Stability

- For short-term and daily use, store the kit at 4°C.
- For long-term storage, it is recommended to make small aliquots of the reagents and store them at -20°C.
- These reagents should be stable until expiration date when stored appropriately at the indicated temperatures.

4. Kit Contents

Contents	100 Tests
2X qPCR Master Mix (UDG)	1 vial (1000 μL/vial)
WSSV Primer Mix	1 vial (300 μL/vial)
ddH_2O	1 vial (1000 μL/vial)
WSSV Postive Standard (10^6 copies /μL)	1 vial (100 μL/vial)
Instruction Manual	1 each

Equipment required not included:

- Micropipette
- Real-Time PCR machine
- High speed centrifuge
- Vortex mixer

5. Detection Limit

This kit enables detection of as low as 10 copies/ μL of target.

6. Precautions

- The test needs to be performed by adequately trained personnel.
- It is recommended to use aerosol-resistant pipette tips to prevent contamination.
- Ensure that all reagents supplied by the kit are completely thawed prior to use.
- When the reagents are thawed, ensure that each reagent is thoroughly mixed by gently tapping onto the tubes.
- Briefly centrifuge the tubes to ensure that the residual substances are collected at the bottom of the tubes.
- Avoid repeated freeze-thaw cycles of the primers and positive standard.
- Prepare the reaction mixture on ice or cooling block.

7. PCR Protocol

1. Thaw the reagents and ensure that they are properly mixed before use.
2. Prepare and label each PCR tube according to the number of samples.

   Note: For each test, positive and negative control should be included

3. Prepare the reaction mixture according to Table 1.

Component	Volume (μL)
2X qPCR Master Mix (UDG)	10.0
WSSV Primer Mix	3.0
ddH_2O	5.0
DNA Template	2.0
Total	20.0

Table 1: PCR reagent mixture table

4. Dispense 18.0 μL of the PCR premix into each labelled PCR tube.

5. Pipette 2.0 μL of the extracted nucleic acid (100~200 ng) into the respective PCR tubes that contain the premix reagent. For negative control, use ddH_2O whereas for positive control, use positive standard provided.

6. Briefly spin the tubes before putting them into the thermal cycler. Perform the PCR amplification according to the conditions stated in this table:

50°C	2 minutes	UDG carry-over prevention	1 cycle
95 °C	10 minutes		1 cycle
95 °C	15 seconds		45 cycles
60 °C	30 seconds	Acquiring on FAM and HEX	

Table 2: PCR amplification cycle

Note: PCR amplification can also be performed using the Multi-test PCR Profile:

50°C	30 minutes	Reverse transcription	1 cycle
95 °C	10 minutes		1 cycle
95 °C	15 seconds		45 cycles
60 °C	30 seconds	Acquiring on FAM and HEX	

Table 3: Multi-test PCR Profile

8. Results and Interpretation

	FAM	HEX	Result
Positive control	+	-	Test is valid
Sample A	+	+	WSSV is detected
Sample B	-	+	WSSV is not detected
Negative control	-	-	Test is valid

Note: Test is considered fail and shall be repeated if:

- FAM signal of Positive control did not pass the threshold.
- FAM signal of Negative control passed the threshold.
- HEX signal is absent for decapods (E.g. shrimp, crab) samples.

9. References

- Durand, S. V., & Lightner, D. V. (2002). Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp. *Journal of Fish Diseases*, *25*(7), 381-389.

10. Troubleshooting

Problem	Cause	Solution
No FAM signal in positive control or sample	Error in collection of fluorescent data	Make sure the correct fluorescent channels are selected and the fluorescent data is collected at the correct step in the protocol setting
	Error in setup	Check the protocol and repeat the reaction
	Reaction components are not mixed completely	Repeat the reaction and make sure the reaction components are mixed well
	Sample degradation	Check the integrity of DNA or RNA sample
No HEX signal in decapods sample	Extraction failure or PCR inhibition	Extraction of the sample should be repeated
Fluorescence signal in negative control or sample	Reagents contaminated	Discard the suspected contaminated reaction components and repeat the assay with fresh reaction components
	Contamination happened during reaction setup	Implement good laboratory practices such as wear glove and lab coat and use barrier tips during reaction setup

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Who we are

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Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

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