HELINI™
White spot Syndrome Virus [WSSV] PCR kit

Instruction manual

Cat. No: 6501-50/100 tests

HELINI WSSV PCR kit

Kit components

No. of reactions	Volume Per reaction	50tests
Red Dye PCR Master mix	10µl	500µl
WSSV Primer Mix	2.5µl	125µl
Endogenous control Primer Mix	2.5µl	125µl
WSSV Positive control	5µl	150µl
PCR grade water		4ml
Handbook		

Storage

Upon arrival of the kit, content of the kit should be stored at –20°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>2 x) should be avoided, as this may reduce assay sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

Storage at 2–8°C should not exceed a period of 5 hours.

Technical Assistance

For technical assistance and more information, please contact;

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Description

HELINI WSSV Semi-nested PCR kit is highly sensitive, ready to use PCR kit for the detection of White spot syndrome virus in Crustaceans using Polymerase chain reactions. [PCR]

Specificity

WSSV primer has been designed for the specific and exclusive *in vitro* detection of WSSV virus. The target sequence (ribonucleotide reductase large subunit) is highly conserved and has previously been shown to be a good genetic marker for WSSV. *[Donald V. Lightner, 2001]* The primers sequence has 100% homology with a broad range of relevant reference sequences based on a comprehensive bioinformatics analysis.

Dynamic range of test

Under optimal PCR conditions, kits have very high priming efficiencies of >95% and can detect less than 3 copies of target template.

Detection Protocol

Things to do before starting

Before each use, all reagents need to be thawed completely, mixed by gently inverting and centrifuged briefly. Make sure that Positive and Negative control is included in every PCR run.

Detection Mix

Components	**Volume**
Red Dye PCR Master Mix	10µl
Endogenous Primer Mix	2.5µl
WSSV Primer Mix	2.5µl
Purified DNA sample*	**2 to 5µl**
Total reaction volume	20µl

*** DNA concentration:**
10ng – 100ng/µl – use 5µl directly. If not able to measure the DNA concentration, try from 2µl to 5µl of DNA per reaction.

Negative Control [NTC]
Add 5µl of sterile PCR grade water.

Positive Control
Add 5µl of Positive control

Centrifuge PCR vials briefly before placing into thermal cycler.

Amplification Protocol

	Step	Time	Temp
	Taq enzyme activation	5min	95ºC
35 cycles	Denaturation	30sec	95ºC
	Annealing	30sec	58ºC
	Extension	30sec	72ºC
	Final extension	5min	72ºC

Expected PCR Product:

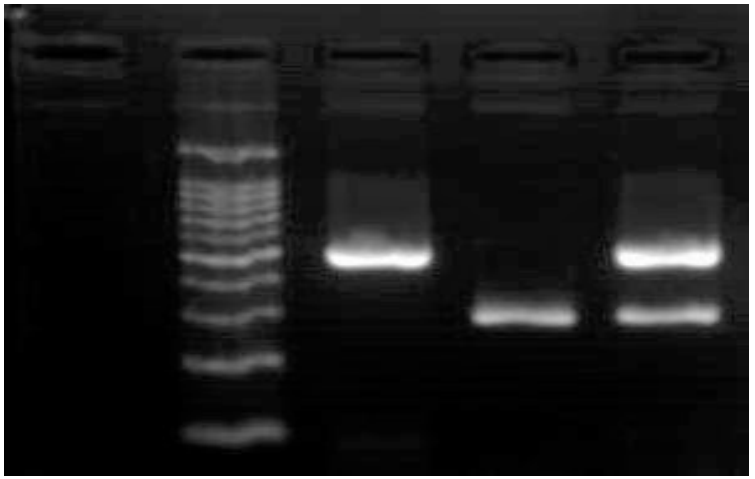
WSSV: 300bp

Endogenous control: 500bp

Agarose gel concentration: 2% agarose gel

Prepare 2.5% agarose gel and load entire PCR product along with 100bp DNA Ladder [Not supplied with the kit]. [PCR Master Mix contains dye and not necessary to add gel loading dye]

Illustration



Lane 1: NTC
Lane 3: Endogenous control
Lane 4: WSSV
Lane 5: Endogenous control + WSSV
Lane 2: DNA Ladder [100bp, 200bp, 300bp, 400bp, 500bp 600bp, 700bp, 800bp, 900bp, 1000bp & 1500bp]

Interpretation:

Lane 3: Assay valid – WSSV DNA NOT detected
Lane 5: Assay valid – WSSV DNA Detected

Test Sample	Negative control	Positive control	Endogenous Control	Interpretation
Positive	Negative	Positive	**Positive**	**Detected**
Negative	Negative	Positive	**Positive**	**Not-Detected**
Negative	Negative	Negative	**Negative**	**Experiment fail**
Positive	Positive	Positive	**Positive**	**Experiment fail**

Vannamei Endogenous control:

Vannamei endogenous control amplification justifies DNA purification efficiency and rule out PCR inhibition.

Limitations

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling.

A false negative result may occur if an excess of DNA template is present in the reaction. If inhibition of the endogenous control is noted for a particular sample, purified DNA can be tested at 2 or more dilutions [e.g., 1:3 and 1:6) to verify the results.

Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.

Manufactured and marketed by

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