

HELINITM

Infectious hypodermal & Hematopoietic necrosis virus [IHHNV] Real-time PCR Kit

Instruction manual

Cat. No: 6502-50/100 tests

Kit components

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

Storage

The 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;

0091-44-24490433

helinibiomolecules@gmail.com

Description

HELINI IHHNV Semi-nested PCR kit is highly sensitive, ready to use PCR kit for the detection of Infectious hypodermal & Hematopoietic necrosis virus [IHHNV] in Crustaceans using Polymerase chain reactions. [PCR]

Specificity

Infectious hypodermal & Hematopoietic necrosis virus [IHHNV] primer has been designed for the specific and exclusive *in vitro* detection of IHHNV virus. The target sequence is highly conserved and has previously been shown to be a good genetic marker for IHHNV. [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 5 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
IHHNV Primer Mix	2.5µl
Purified DNA sample*	2 to 5µl
Total reaction volume	20µl

* DNA concentration:

100ng – 500ng/reaction

If not able to measure the DNA concentration, try from 2µl to 5µl of DNA per reaction.

Centrifuge PCR vials briefly before placing into thermal cycler.

Negative Control [NTC]

Add 5µl of sterile PCR grade water.

Positive Control

Add 5µl of Positive control

Amplification Protocol

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

Expected PCR Product:

IHHNV: 380bp

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]

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

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

HELINI *Biomolecules*,
Ohmlina, 26, 2nd Avenue,
Khuthubi Complex, Vettuvankeni,
Chennai 600115, Tamilnadu, INDIA
www.helinibiomolecules.com
www.helini.in
helinibiomolecules@gmail.com
+91-44-24490433

