HELINI™ Enterocytozoon hepatopenaei [EHP] PCR kit

Instruction manual

Cat. No: 6503-50/100tests

HELINI EHP PCR kit

Kit components

No. of reactions	Volume Per reaction	50tests
Red Dye PCR Master mix	10µl	500µl
EHP Primer Mix	2.5µl	125µl
Endogenous control Primer Mix	2.5µl	125µl
EHP 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;

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Description

HELINI EHP PCR kit is high sensitive, ready to use PCR kit for the detection of Enterocytozoon hepatopenaei in Crustaceans using Polymerase chain reactions. [PCR]

Specificity

Enterocytozoon hepatopenaei [EHP] primer has been designed for the specific and exclusive *in vitro* detection of EHP. The target sequence (ribosomal RNA) is highly conserved and has previously been shown to be a good genetic marker for EHP. *[Amornrat Tangprasittipap & et al, Mahidol University]* 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
Vannamei Endogenous Primer Mix	2.5µl
EHP 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 nuclease free water.

Positive Control

Add 5µl of Positive control

Amplification Protocol

	Step	Time	Temp
	Taq enzyme activation	5min	95ºC
35 cycles	Denaturation	30sec	95ºC
	Annealing	30sec	58ºC
	Extension	45sec	72ºC
	Final extension	5min	72ºC

Expected PCR Product:
EHP: 370bp
Vannamei Endogenous control: 500bp

Agarose gel concentration: 2% agarose gel

Prepare 2% agarose gel and load entire PCR product along with 100bp DNA Ladder [Not provided in 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**

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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