REAL-TIME PCR KIT FOR ENTEROCYTOZOON HEPATOPENAEI (EHP) DETECTION

-Intended for molecular detection of EHP in shrimp

CATALOG NUMBER: DIG-EHPR-25

For Research Use Only

Contents

Kit Contents

Components	Volume
Master Mix with ROX (2X)	300 µL×1 vial
EHP Primer & Probe Mix [ready to use]	50 µL × 1 vial
Internal control primer probe mix [ready to use]	50 µL × 1 vial
EHP Positive control	60 µL × 1 vial
Nuclease free water	200 µL × 1 vial

Storage

The contents of the kit should be stored at –20 °C and are stable until the expiration date. 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. The master mix may be frozen at -20 ℃ for long term storage. Once thawed, it should be stored at 4 ℃ and used within 6 months.

Precaution before use

1. Before use, make sure the master mix is evenly mixed by gently inverting the tube several times without creating bubbles. Uneven reagent mixing will result in inadequate reactivity. Do not mix by vortexing.
2. When stored frozen at -20 ℃, master mix may precipitate. To dissolve the precipitate completely, warm by hand or let stand at room temperature briefly, then invert the tube several times. Make sure the reagents are evenly mixed before use.
3. Place reagents on ice immediately after it has thawed.
4. Use fresh disposable tips to minimize potential cross-contamination between samples when preparing reaction mixtures or dispensing aliquots.

Equipment and materials to be supplied by User

- PCR tubes or plates designed specifically for the qPCR instrument
- Micropipette and tips (sterile, with filter)
- Real-time PCR thermal cycler
- Ice bucket

Introduction

The aquaculture industry is one of the fastest-growing food production sectors and constitutes an important economic activity in developing countries. However, climate change and the emergence of pathogens have negatively impacted the aquaculture industry over the past 20 years. Infectious diseases caused by viruses, bacteria, fungi, and microsporidia pose a serious threat to shrimp aquaculture. Among them, viral diseases are by far the most difficult to manage.

Product Description

The Real-time PCR kit for Enterocytozoon hepatopenaei (EHP) is intended for the molecular detection of EHP in shrimp. Hepatopancreatic microsporidiosis (HPM) is a disease caused by infection with *Enterocytozoon hepatopenaei* (EHP), a microsporidian pathogen. EHP belongs to the family *Enterocytozoonidae*, phylum Microsporidia, which comprises highly reduced and specialized, spore-forming, unicellular parasites of animals, including humans, insects and crustaceans. Externally visible clinical signs are often used for presumptive diagnosis of suspected infections in shrimp. In the case of EHP, however, externally visible signs are often absent, apart from retarded growth over time. This assay allows rapid detection of EHP which involves the use of target and internal control sets of primers in a single tube by using real-time polymerase chain reaction.

Protocol

- For all reagents preparation, wear appropriate gloves.

- A Master Mix is prepared in a tube by combining a 2X Master Mix Reagent and Primer & Probe Mix with water (protect from light).

Reagents	Vol. for 1 rxn (µL)
Water	4.8
Master Mix with ROX (2X)	10
EHP Primer & Probe Mix [ready to use]	1.6
Internal control Primer probe mix [ready to use]	1.6
Template DNA/Positive control	2.0
Total	20

- TaqMan® probe reporter dyes and quenchers

Detector Name	Reporter	Quencher
EHP Target	FAM™ dye	None
Internal Control	VIC™ dye	None

- After mixing thoroughly, 18µl volumes of the Master Mix are quickly dispensed into the plate/strip.
- Then add 2µl of DNA test samples and controls.
- Pulse spin tubes at 3000rpm.
- Keep the plate/strips/tubes in thermal cycler

Stages		Cycles	Temperature	Time
Template denaturation	Holding stage	1	95 °C	30 sec
Amplification	Cycling stage	40	95 °C 60 °C	5 sec 31 sec

- After the completion of the reaction, observe the Ct value of the samples and controls.

Specificity

The primers have been designed for specific and *in vitro* detection of EHP. This kit has high priming efficiency of ≥ 95 % and can detect less than 10 copies of target template.

Expected Ct value: Ct < 38 – Positive

- In positive control (PC), only target shows Ct value less than 38 whereas no Ct value is observed in endogenous control gene of PC.
- For samples, both target and endogenous control gene shows Ct value less than 38 which is considered as positive.

Data interpretation:

	Outcome 1	Outcome 2	Outcome 3	Outcome 4
Test sample	Positive	Negative	Positive	Negative
Positive control	Positive	Positive	Positive	Negative
Negative control	Negative	Negative	Positive	Negative
Internal control	Positive	Positive	Positive	Negative
Interpretation	Infection detected	Infection not detected	PCR Contamination	Experiment failed

Troubleshooting guide

- Setup errors
  - Mix well all reagents before every use.
  - Make master mixes whenever possible as this reduces pipetting errors.
- Fluorescence in negative control
  - There is a possibility that template DNA might be added in the NTC reaction.
  - Contamination in reagents-Aliquot the reagents before setting up the reaction.
  - Random contamination
    - Clean the work area with 10% bleach and nuclease-free water
    - Use separate working areas for PCR mix preparation, template addition, and performing the PCR reactions.
- Unexpected data values
  - Samples incorrectly labelled-Rerun samples or plate using extra caution when loading
  - Inhibitors in sample-Run a dilution of samples (inhibitors will be diluted and may result in lower C_q for diluted material)
- C_q is much earlier than anticipated- Genomic DNA is contaminated with RNA
  - Test assay performance against carefully quantified controls
- Jagged signal throughout amplification plot- Poor amplification or weak probe signal
  - Ensure that a sufficient amount of probe is used so that background noise isn't amplified
  - Try a fresh batch of probe
  - Mix primer/probe/master solution thoroughly during reaction set up
- Rough amplification curves
  - Improper storage of primers and probes can cause them to degrade and lose specificity, which in turn affects the reaction efficiency.

TECHNICAL ASSISTANCE

For technical assistance and enquiries

Contact No: +91 9566150904

Email id: info.diagnomics@gmail.com

Manufactured and marketed by

Diagnomics (OPC) Pvt Ltd
Agri-Business Incubator, ICAR-CIBA,
ICAR-Central Institute of Brackish water Aquaculture
Indian Council of Agricultural Research,
75, Santhome High Road, MRC Nagar,
Chennai 600 028, Tamil Nadu, India
Mobile: +91 9566150904, +91 9003484895
Email: info.diagnomics@gmail.com