User Guide

Qexp-Aqu Shrimp Disease Detection Kit-IHHNV

Research Use Only

A. Sample Collection and Lysis

Tissue

1. Take 10-20 mg of shrimp hepatopancreas and place it into a 1.5 mL tube.
2. Homogenize the tissue with a grinding rod*.
3. Add 600 µL lysis buffer into the tube and rinse the grinding rod*.
4. Mix lysate by vortex or pipetting, and then transfer 200 µL lysate into a 0.2 mL PCR tube.
5. Incubate the lysate at 95°C for 15 minutes, then cool it to room temperature.
6. Transfer 20 µL lysate to a new PCR tube and add 180 µL nuclease-free water and gently mix by vortex or pipetting **(0.1X lysate sample)**.

*You could also use an alternate tool to grind the tissue and release the nucleic acid.

Shrimp larvae

1. Take 10-20 mg of shrimp larvae (<1 cm) into a 1.5 mL tube.
2. Homogenize the shrimp larvae with a grinding rod*.
3. Add 600 µL of lysis buffer into the tube and rinse the grinding rod*.
4. Mix lysate by vortex or pipetting, and then transfer 200 µL lysate into a 0.2 mL PCR tube.
5. Incubate the lysate at 95°C for 15 minutes, then cool it to room temperature.
6. Transfer 20 µL lysate to a new PCR tube and add 180 µL nuclease-free water and gently mix by vortex or pipetting **(0.1X lysate sample)**.

*You could also use an alternate tool to grind the tissue and release the nucleic acid.

B. Direct PCR Master Mix Preparation

1. Prepare direct PCR master mix reagents as shown in the table below:

Component	Volume per sample (µL)	Volume for n samples plus 2 controls (µL)
Primer mix-IHHNV	10	10 x (n+2)
2XDirectGO PreMix	12.5	12.5 x (n+2)
TOTAL (µL)	22.5	22.5 x (n +2)

2. Transfer 22.5 µL Master Mix into each of the 0.2 mL PCR tubes after mixing it gently.

C. **PCR Process**

1. Add samples as shown in the table below:

Component	Volume per reaction		
	NTC	Positive control	sample
Direct PCR Master Mix	22.5	22.5	22.5
Nuclease-free water	2.5	-	-
Positive control	-	2.5	-
0.1X lysate sample	-	-	2.5
TOTAL (μL)	25	25	25

D. **PCR Program Set-up**

Step	Number of cycles	Temperature (℃)	Time
Activation	1	95	10 minutes
Denaturation	40	95	20 seconds
Annealing		60	20 seconds
Extension		72	20 seconds
Final Extension	1	72	2 minutes
Hold	1	14	hold

E. **Analysis and Results**

Using BiOptic *Qsep* series Bio-Fragment Analyzers to analyze the PCR amplicon

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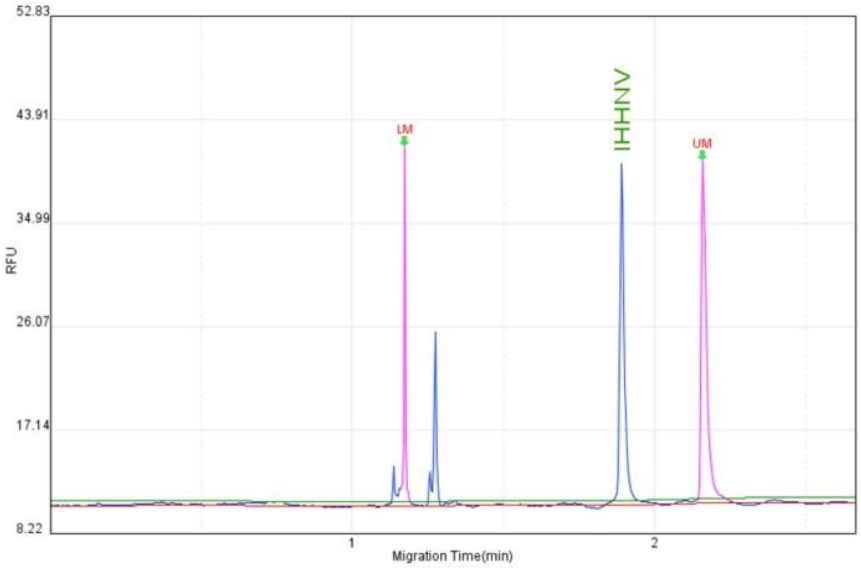
	IHHNV
Target size	340 bp

The result is shown below: