User Guide

Qexp-Aqua Shrimp Disease Detection Kit-AHPND

Research Use Only

A. Sample Collection and Lysis

Determine the appropriate sampling method based on the shrimp sizes specified in the provided table:

Shrimp size	Sampling Type	Sampling Count
< 1cm	Whole shrimp	3 shrimp
1cm-2cm	Shrimp head	3 shrimp head
2cm-5cm	Hepatopancreas	1 hepatopancreas
5cm-10cm	Hepatopancreas	½ hepatopancreas
>10cm	Hepatopancreas	¼ hepatopancreas

Hepatopancreas Sample:

1. Carefully extract the hepatopancreas (HP) from the shrimp using tweezers and place it into a 1.5 mL tube.
2. Homogenize the tissue with a grinding rod*.
3. Take a new 0.2 mL PCR tube and add 90 µL of DNA lysis buffer.
4. Transfer 3 µL of the homogenized HP tissue into the PCR tube and gently mix the lysate by pipetting.
5. Incubate the lysate at 95°C for 15 minutes, then cool it to room temperature.
6. Transfer 10 µL lysate into a new PCR tube, add 90 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 Sample:

1. Take 3 shrimp larvae (<1 cm) or shrimp heads (1-2 cm) into a 1.5 mL tube.
2. Homogenize the shrimp larvae with a grinding rod*.
3. Add 400 µL of DNA lysis buffer into the tube and rinse the grinding rod*.
4. Mix lysate by pipetting, then transfer 90 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 10 µL lysate to a new PCR tube, add 90 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. 1st Direct PCR Mix Preparation

1. Prepare 1st Direct PCR Mix reagents as shown in the table below:

Component	Volume per sample (µL)	Volume for n samples plus 1 NTC (µL)
Primer Mix 1-ANDPH	12	12 x (n+1)
Direct PCR PreMix	15	15 x (n+1)
TOTAL (µL)	27	27 x (n +1)

2. Transfer 27 µL 1st Direct PCR Mix into each of the 0.2 mL PCR tubes after mixing it gently.

C. 1st PCR Process

Add samples as shown in the table below:

Component	Volume per reaction	
	NTC	sample
1st Direct PCR Mix	27	27
Nuclease-free water	3	-
0.1X lysate sample	-	3
TOTAL (µL)	30	30

D. 1st PCR Program Set-up

Step	Number of cycles	Temperature (℃)	Time
Activation	1	95	10 minutes
Denaturation	20	95	30 seconds
Annealing		62	30 seconds
Extension		72	40 seconds
Final Extension	1	72	5 minutes
Hold	1	14	hold

E. 2nd Direct PCR Mix Preparation

1. Prepare 2nd Direct PCR Mix reagents as shown in the table below:

Component	Volume per sample (µL)	Volume for n samples plus 2 controls (µL)
Primer Mix 2-ANDPH	12	12 x (n+2)
Direct PCR PreMix	15	15 x (n+2)
TOTAL (µL)	27	27 x (n +2)

2. Transfer 27 µL 2nd Direct PCR Mix into each of the 0.2 mL PCR tubes after mixing it gently.

F. 2nd PCR Process

1. Transfer 10 µL 1st PCR Product including Negative Control (Step D) to a new PCR tube, add 90 µL nuclease-free water, and gently mix by vortex or pipetting (0.1X 1st PCR Product/ 1st Negative Control).
2. Add samples as shown in the table below:

Component	Volume per reaction		
	NTC	Positive control	sample
2nd Direct PCR Mix	27	27	27
0.1X 1st Negative Control	3	-	-
Positive control	-	3	-
0.1X 1st PCR Product	-	-	3
TOTAL (µL)	30	30	30

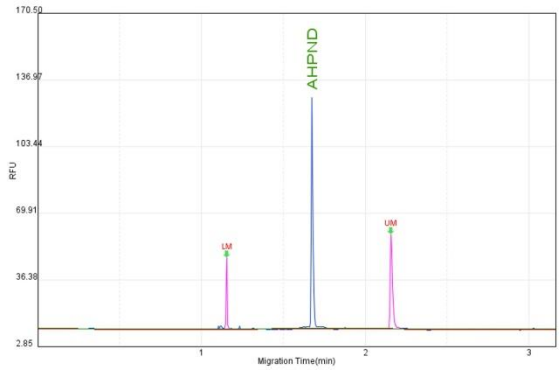
G. 2nd PCR Program Set-up

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	5 minutes
Hold	1	14	hold

H. Analysis and Results

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

Cartridge	Method	Alignment Marker
S2	M-4-10-08-160	20-1K

The result is shown below:

	AHPND
Target size	180 bp