

[Cat. No.] **K-2988**

IPC Forward primer	0.2 µM
IPC Reverse primer	0.2 µM
IPC Probe (Cy5)	0.1 µM

Introduction

AccuPower® Shrimp Disease 2 Real-Time PCR Kit is can detect 4 diseases (Acute Hepatopancreatic Necrosis Disease, *Enterocytozoon hepatopenae* infection, White syndrome disease, Infectious myonecrosis) that infect shrimp.

Clinical signs include white spots on the epidermis or poor feeding, empty stomach, partial firmness, or white lesions on the muscles, resulting in death. These pathogens are causing huge economic losses in shrimp farming Vietnam, Malaysia and Thailand, as well as in several Asian countries.

In this product, all elements (RTase, DNA polymerase, primers, dNTPs, reaction buffer) necessary for real-time PCR of 4 pathogens simultaneously or specifically are dried in a PCR tube, so the user can only add template DNA/RNA, internal positive control (IPC) and DEPC-D.W. You can easily prepare a real-time PCR reaction solution.

Applications

- Qualitative analysis of multiplex real-time PCR for AHPND, EHP, IMNV, WSSV pathogen with Internal Positive Control (IPC).

Components

Components	Amount
PreMix	8-strips x 12 ea
Positive Control (2x10 ⁷ copies/µl)	50 µl
Internal Positive Control (1x10 ⁵ copies/µl)	100 µl
Sealing film	1 ea
DEPC-D.W.	1 ea

* **Note:** For research use only. Not for use in diagnostic or therapeutic procedures.

Composition

Composition	50 µl reaction	
PreMix	RocketScript™ Reverse transcriptase	1 U
	Taq DNA Polymerase	6 U
	dNTPs (dATP, dCTP, dGTP, dTTP)	Each 300 µM
	Reaction buffer with 2 mM MgCl ₂	1X
Oligo	AHPND Forward primer	0.4 µM
	AHPND Reverse primer	0.4 µM
	AHPND Probe (TET)	0.25 µM
	EHP Forward primer	0.6 µM
	EHP Reverse primer	0.6 µM
	EHP Probe (FAM)	0.3 µM
	IMNV Forward primer	0.4 µM
	IMNV Reverse primer	0.4 µM
	IMNV Probe (Texas Red)	0.15 µM
	WSSV Forward primer	0.2 µM
	WSSV Reverse primer	0.2 µM
	WSSV Probe (TAMRA)	0.1 µM

Specifications

Taq DNA Polymerase	
5'→3' exonuclease activity	Yes
3'→5' exonuclease activity	No
3'-A overhang	Yes

Storage

Store at -20°C. If stored in the recommended temperature, this product will be stable until the expiration date printed out on the label.

Online Resources



Visit our **product page** for additional information and protocols.

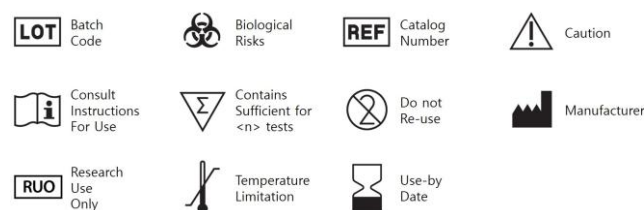
Ordering Information

Description	Cat. No.
AccuPower® Shrimp Disease 2 Real-Time PCR Kit, Exicycler 8-well strips / 96 tubes	K-2988




Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols



Experimental Procedures

Steps		Procedure Details																																			
1	 Preparation of reaction mixture	<p>1. Prepare <i>AccuPower</i>® Shrimp Disease 2 Real-Time PCR Kit, template DNA/RNA, Internal Positive Control DNA and DEPC-DW.</p>																																			
2	 Composition of reaction mixture	<p>2. Add all components into PCR tubes referring to the following list of components (based on 1 test).</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;">Components</th> <th style="text-align: center;">Volume (µl)</th> </tr> </thead> <tbody> <tr> <td>Internal Positive Control DNA</td> <td style="text-align: center;">1</td> </tr> <tr> <td>Template DNA/RNA (Positive Control)</td> <td style="text-align: center;">5</td> </tr> <tr> <td>DEPC-DW</td> <td style="text-align: center;">Up to 50</td> </tr> <tr> <td>Total reaction volume</td> <td style="text-align: center;">50</td> </tr> </tbody> </table> <p>(The volume of the premix dried in the PCR tube is not included.)</p> <p>3. Vortex the reaction solution to completely melt the PreMix, then spin down.</p>	Components	Volume (µl)	Internal Positive Control DNA	1	Template DNA/RNA (Positive Control)	5	DEPC-DW	Up to 50	Total reaction volume	50																									
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3	 Real-time PCR	<p>4. After installing the PCR tube in the <i>Exicycler</i>, set the PCR conditions as follows.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;">Step</th> <th style="text-align: center;">Temperature</th> <th style="text-align: center;">Time</th> <th style="text-align: center;">Cycles</th> </tr> </thead> <tbody> <tr> <td>Reverse Transcription</td> <td style="text-align: center;">50 °C</td> <td style="text-align: center;">15 min</td> <td style="text-align: center;">1 cycle</td> </tr> <tr> <td>Pre-denaturation</td> <td style="text-align: center;">95 °C</td> <td style="text-align: center;">5 min</td> <td style="text-align: center;">1 cycle</td> </tr> <tr> <td>Denaturation</td> <td style="text-align: center;">95 °C</td> <td style="text-align: center;">5 sec</td> <td rowspan="2" style="text-align: center;">45 cycles</td> </tr> <tr> <td>Annealing& Extension</td> <td style="text-align: center;">55 °C</td> <td style="text-align: center;">30 sec</td> </tr> <tr> <td>Scan</td> <td></td> <td></td> <td></td> </tr> </tbody> </table> <p>* Note: Users can adjust the protocol according to their instrument and template sequences to get optimal results.</p> <ul style="list-style-type: none"> Perform real-time PCR by selecting a total of 5 types of fluorescence. <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;">Target</th> <th style="text-align: center;">Flourescence</th> </tr> </thead> <tbody> <tr> <td>AHPND</td> <td style="text-align: center;">TET</td> </tr> <tr> <td>EHP</td> <td style="text-align: center;">FAM</td> </tr> <tr> <td>IMNV</td> <td style="text-align: center;">Texas Red</td> </tr> <tr> <td>WSSV</td> <td style="text-align: center;">TAMRA</td> </tr> <tr> <td>Internal Positive Control</td> <td style="text-align: center;">Cy5</td> </tr> </tbody> </table> <p>5. After the reaction is completed, analyze the results.</p>	Step	Temperature	Time	Cycles	Reverse Transcription	50 °C	15 min	1 cycle	Pre-denaturation	95 °C	5 min	1 cycle	Denaturation	95 °C	5 sec	45 cycles	Annealing& Extension	55 °C	30 sec	Scan				Target	Flourescence	AHPND	TET	EHP	FAM	IMNV	Texas Red	WSSV	TAMRA	Internal Positive Control	Cy5
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