

[Cat. No.] **K-2925**

IMNV Probe (Texas Red)	0.3 µM
IPC Forward primer	0.3 µM
IPC Reverse primer	0.3 µM
IPC Probe (Cy5)	0.3 µM

### Introduction

AccuPower® Shrimp Disease Real-Time PCR Kit can simultaneously detect major diseases infecting shrimp (White syndrome disease, yellow head disease, Taura syndrome, Infectious myonecrosis) through real-time polymerase chain reaction (Real-time PCR).

Clinical signs of the disease include loss of appetite, white spots on the epidermis, and light yellow and hardened hair. It can also cause white lesions to develop in the muscles of severely affected individuals, leading to death. These diseases are causing enormous economic losses to shrimp farming in Vietnam, Malaysia, Thailand, as well as many other 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 WSSV, YHV-1, TSV, IMNV pathogen with Internal Positive Control (IPC).

### Components

Components	Amount
PreMix	8-strips x 12 ea
Positive Control (2x10 <sup>7</sup> copies/µl)	50 µl
Internal Positive Control (1x10 <sup>5</sup> copies/µl)	100 µl
Sealing film	1 ea
DEPC-D.W.	1.3 ml

\* 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 <sub>2</sub>	1X
Oligo	
WSSV Forward primer	0.4 µM
WSSV Reverse primer	0.4 µM
WSSV Probe (TAMRA)	0.4 µM
YHV-1 Forward primer	0.2 µM
YHV-1 Reverse primer	0.2 µM
YHV-1 Probe (TET)	0.2 µM
TSV Forward primer	0.2 µM
TSV Reverse primer	0.2 µM
TSV Probe (FAM)	0.2 µM
IMNV Forward primer	0.3 µM
IMNV Reverse primer	0.3 µM

\* Note: Primers for WSSV and T detection are from Durand S.V. and Lightner D.V (2002), Tang K.F.J et al., (2004) as primer for TSV detection, and Andrade T.P.D et al., (2007) as primer for IMNV detection.

### 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.

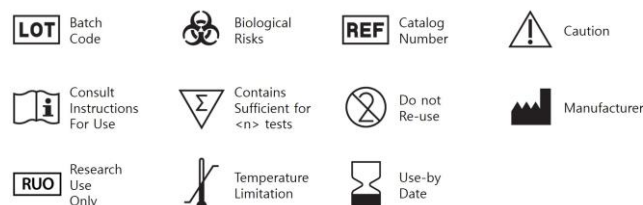
### Ordering Information

Description	Cat. No.
AccuPower® Shrimp Disease Real-Time PCR Kit, Exicycler 8-well strips / 96 tubes	K-2925




### Notice

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### Explanation of Symbols



**Experimental Procedures**

Steps		Procedure Details																																				
1	 <b>Preparation of reaction mixture</b>	<p>1. Prepare <i>AccuPower</i>® Shrimp Disease Real-Time PCR Kit, template DNA/RNA, Internal Positive Control DNA and DEPC-DW.</p>																																				
2	 <b>Composition of reaction mixture</b>	<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;">1~5</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)	1~5	DEPC-DW	Up to 50	Total reaction volume	50																										
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3	 <b>Real-time PCR</b>	<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;">10 sec</td> <td style="text-align: center;">45 cycles</td> </tr> <tr> <td>Annealing&amp; Extension</td> <td style="text-align: center;">55 °C</td> <td style="text-align: center;">20 sec</td> <td></td> </tr> <tr> <td>Scan</td> <td></td> <td></td> <td></td> </tr> </tbody> </table> <ul style="list-style-type: none"> <li>Perform real-time PCR by selecting a total of 5 types of fluorescence.</li> </ul> <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>WSSV</td> <td style="text-align: center;">TAMRA</td> </tr> <tr> <td>YHV-1</td> <td style="text-align: center;">TET</td> </tr> <tr> <td>TSV</td> <td style="text-align: center;">FAM</td> </tr> <tr> <td>IMNV</td> <td style="text-align: center;">Texas Red</td> </tr> <tr> <td>Internal Positive Control</td> <td style="text-align: center;">Cy5</td> </tr> </tbody> </table>	Step	Temperature	Time	Cycles	Reverse Transcription	50 °C	15 min	1 cycle	Pre-denaturation	95 °C	5 min	1 cycle	Denaturation	95 °C	10 sec	45 cycles	Annealing& Extension	55 °C	20 sec		Scan				Target	Flourescence	WSSV	TAMRA	YHV-1	TET	TSV	FAM	IMNV	Texas Red	Internal Positive Control	Cy5
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