

[Cat. No.] **K-2914**

### Introduction

AccuPower® RSIV/ISKNV Multiplex Master Mix is a product which can detect red sea bream iridovirus (RSIV) and infectious kidney and spleen necrosis virus (ISKNV), the causative agents of red sea bream iridoviral disease (RSIVD) in fish through real-time polymerase chain reaction (real-time PCR). Red sea bream iridoviral disease (RSIVD) causes mass mortality in more than 30 species of fish, mainly sea bream, amberjack, mackerel, etc. Clinical symptoms of RSIVD include lethargy, severe anemia, gill petechiae, and spleen enlargement. RSIVD is an aquatic disease which consistently occurs in East and South-East Asian countries.

This product contains all real-time PCR components specific to RSIV and ISKNV, including RTase, DNA polymerase, primers, dNTPs, and reaction buffer. The users can easily prepare a reaction mixture simply by adding template DNA, internal positive control (IPC), oligo mix, and DEPC-D.W.

### Features & Benefits

- Convenience: All necessary reactants for real-time PCR are included in a tube (i.e., Master Mix type), allowing the users to perform reaction simply by adding template DNA, oligo mix, and DEPC-D.W.
- Sensitivity: By using BIONEER's PyroHotStart RT reaction and HotStart Taq DNA Polymerase that minimizes non-specific reactions and maximizes reaction efficiency, only the target gene can be effectively amplified even with a trace amount of template DNA.

### Components

Components	Amount
Master Mix	1.5 ml
Oligo Mix	400 µl
Positive Control (2x10 <sup>7</sup> copies/µl)	50 µl
Internal Positive Control (1x10 <sup>5</sup> copies/µl)	100 µl
PC Dilution Buffer	1 ml
DEPC-DW	1.3 ml

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

### Composition

Composition	25 µl reaction
<i>RocketScript™</i> Reverse transcriptase	1 U
Taq DNA polymerase	6 U
dNTPs (dATP, dCTP, dGTP, dTTP)	Each 150 µM
Reaction buffer with 2 mM MgCl <sub>2</sub>	1X
RSIV Forward primer	0.6 µM
RSIV Reverse primer	0.6 µM
RSIV Probe (FAM)	0.6 µM
ISKNV Forward primer	0.4 µM
ISKNV Reverse primer	0.4 µM
ISKNV Probe (Texas Red)	0.4 µM

IPC Forward primer	0.4 µM
IPC Reverse primer	0.4 µM
IPC Probe (Cy5)	0.4 µM
ROX Dye	1X

### 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® RSIV/ISKNV Multiplex Master Mix, 1.5 ml of Master Mix solution, 100 tests	K-2914




### 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

Batch Code	Biological Risks	Catalog Number	Caution
Consult Instructions For Use	Contains Sufficient for <n> tests	Do not Re-use	Manufacturer
Research Use Only	Temperature Limitation	Use-by Date	

**Experimental Procedures**

Steps		Procedure Details															
1	 <b>Preparation of reaction mixture</b>	<p>1. Before use, thaw all components of <i>AccuPower®</i> RSIV/ISKNV Multiplex Master Mix on ice and mix them thoroughly. Then, briefly spin down all components.</p>															
2	 <b>Composition of reaction mixture</b>	<p>2. Add all components into PCR tubes or a plate 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: left;">Components</th> <th style="text-align: right;">Volume (µl)</th> </tr> </thead> <tbody> <tr> <td>Master Mix</td> <td style="text-align: right;">15</td> </tr> <tr> <td>Oligo Mix</td> <td style="text-align: right;">4</td> </tr> <tr> <td>Template DNA (Positive Control)</td> <td style="text-align: right;">5</td> </tr> <tr> <td>Internal Positive Control</td> <td style="text-align: right;">1</td> </tr> <tr> <td><b>Total volume</b></td> <td style="text-align: right;"><b>25</b></td> </tr> </tbody> </table>	Components	Volume (µl)	Master Mix	15	Oligo Mix	4	Template DNA (Positive Control)	5	Internal Positive Control	1	<b>Total volume</b>	<b>25</b>			
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3	 <b>Real-time PCR</b>	<p>3. Place PCR tubes or a plate on the real-time quantitative thermal cycler.</p> <p>4. Perform the reaction under the following conditions.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left;">Step</th> <th style="text-align: left;">Temperature</th> <th style="text-align: left;">Time</th> <th style="text-align: left;">Cycles</th> </tr> </thead> <tbody> <tr> <td>Pre-denaturation</td> <td>95°C</td> <td>5 min</td> <td>1 cycle</td> </tr> <tr> <td>Denaturation</td> <td>95°C</td> <td>10 sec</td> <td rowspan="2">45 cycles</td> </tr> <tr> <td>Annealing &amp; Extension</td> <td>55°C</td> <td>20 sec</td> </tr> </tbody> </table> <p>* <b>Note:</b> Users can adjust the protocol according to their instrument and template sequences to get optimal results.</p> <p>5. After the reaction is completed, analyze the results.</p>	Step	Temperature	Time	Cycles	Pre-denaturation	95°C	5 min	1 cycle	Denaturation	95°C	10 sec	45 cycles	Annealing & Extension	55°C	20 sec
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