

[Cat. No.] **K-2991**

Introduction

AccuPower[®] Salmon Disease Multiplex Real-Time PCR Kit is a PCR product that can detect 4 diseases (Infectious Pancreatic Necrosis Virus (IPNV), Infectious Salmon Anaemia Virus (ISAV), Bacterial Kidney Disease (BKD), Salmonid Rickettsial Septicaemia (SRS)) that infect salmon. Clinical signs of four diseases include swollen abdomen or eyes or darkening of the skin, pale gills, haemorrhage at the base of the fins, lesions of the skin and death in acute cases. These infectious diseases are causing and increasing huge economic losses in salmon farming industry especially Chile, as well as in Canada, Ireland, Scotland and Norway.

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 tube, so the user can only add template DNA/RNA and DEPC-D.W. You can easily prepare a PCR reaction solution.

Applications

- Qualitative analysis of multiplex real-time PCR for IPNV, ISAV, BKD, SRS pathogen.

Components

Components	Amount
PreMix	8-strips x 12 ea
Positive Control (1x10 ⁸ copies/μl)	50 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	<i>RocketScript</i> [™] Reverse transcriptase	1 U
	<i>Taq</i> DNA Polymerase	4 U
	dNTPs (dATP, dCTP, dGTP, dTTP)	Each 300 μM
	Reaction buffer with 2 mM MgCl ₂	1X
Oligo	IPNV Forward primer	0.3 μM
	IPNV Reverse primer	0.3 μM
	IPNV Probe (FAM)	0.3 μM
	ISAV Forward primer	0.45 μM
	ISAV Reverse primer	0.45 μM
Oligo	ISAV Probe (Cy5)	0.45 μM
	BKD Forward primer	0.3 μM

BKD Reverse primer	0.3 μM
BKD Probe (Texas Red)	0.3 μM
SRS Forward primer	0.45 μM
SRS Reverse primer	0.45 μM
SRS Probe (TET)	0.45 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.
<i>AccuPower</i> [®] Salmon Disease Multiplex Real-Time PCR Kit, <i>Exicycler</i> 8-well strips / 96 tubes	K-2991

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	 Preparation of reaction mixture	<p>1. Prepare AccuPower® Salmon Disease Multiplex Real-Time PCR Kit, template DNA /RNA, and DEPC-D.W.</p>																																	
2	 Composition of reaction mixture	<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: center;">Components</th> <th style="text-align: center;">Volume (µl)</th> </tr> </thead> <tbody> <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)	Template DNA/RNA (Positive control)	1~5	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;">10 sec</td> <td rowspan="2" style="text-align: center;">45 cycles</td> </tr> <tr> <td>Annealing& Extension</td> <td style="text-align: center;">60 °C</td> <td style="text-align: center;">20 sec</td> </tr> <tr> <td colspan="4">Scan</td> </tr> </tbody> </table> <ul style="list-style-type: none"> Perform real-time PCR by selecting a total of 4 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 style="text-align: center;">IPNV</td> <td style="text-align: center;">FAM</td> </tr> <tr> <td style="text-align: center;">ISAV</td> <td style="text-align: center;">Cy5</td> </tr> <tr> <td style="text-align: center;">BKD</td> <td style="text-align: center;">Texas_Red</td> </tr> <tr> <td style="text-align: center;">SRS</td> <td style="text-align: center;">TET</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	10 sec	45 cycles	Annealing& Extension	60 °C	20 sec	Scan				Target	Flourescence	IPNV	FAM	ISAV	Cy5	BKD	Texas_Red	SRS	TET
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