AccuPower® AHPND PCR Kit

[Cat. No.] K-2923

Introduction

AccuPower® AHPND PCR Kit is a ready-to-use premix for nested PCR that can be used to detect shrimp disease Acute hepatopancreatic necrosis disease (AHPND).
Vibrio parahaemolyticus is the causative agent of AHPND which contains toxic plasmids: *PirA* and *PirB*. Infected shrimps may experience slow growth, empty stomach, whitened liver and pancreas, and softened shell, eventually leading to death. Hence, AHPND may cause serious economic losses to the shrimp aquaculture industry. In major countries such as China, Vietnam, Malaysia, and Thailand that grow shrimp, the mortality rate during the outbreak is more than 80%, leading to a decrease in the global shrimp supply.
This product contains vacuum-dried components specific to *Vibrio parahaemolyticus* including DNA polymerase, primers, dNTPs, and reaction buffer required for PCR. This ready-to-use kit simplifies preparation of PCR mixture as the user only has to add template DNA and nuclease-free water. After the reaction, since tracking dye is included, the samples can be applied directly on agarose gel for analysis without adding extra solution.

Features & Benefits

- Nested PCR: Detect AHPND with nested PCR consisting of AHPND 1st PCR Kit and AHPND 2nd PCR Kit.
- Convenience & Reproducibility: All reactants necessary for PCR including primers are lyophilized in each PCR tube, providing reproducible results in a convenient way.
- Sensitivity: By applying the patented PyroHotStart (Enzyme-mediated HotStart) technology 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.
- Stability: Included stabilizer in the PCR reaction mixture provides increased stability compared to solution-type products.

Composition

Composition	20 µl reaction	
	1st PCR	2nd PCR
Top DNA Polymerase	1 U	1 U
dNTPs (dATP, dCTP, dGTP, dTTP)	Each 250 µM	Each 250 µM
Reaction buffer with 1.5 mM $MgCl_2$	1X	1X
Stabilizer and tracking dye	O	O
AP4-F1 Forward primer	0.5 µM	X
AP4-R1 Reverse primer	0.5 µM	X
AP4-F2 Forward primer	X	0.5 µM
AP4-R2 Reverse primer	X	0.5 µM

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

Specifications

Top DNA Polymerase	
5'→3' exonuclease activity	No
3'→5' exonuclease activity	No
3'–A overhang	Yes
1st fragment size	1,269 bp
2nd fragment size	230 bp

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

English

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

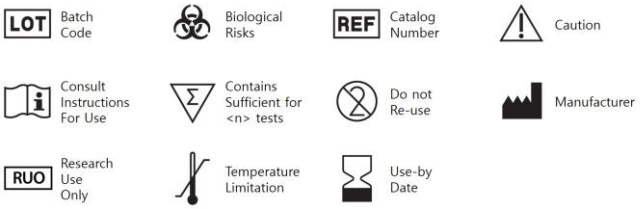
Ordering Information

Description	Cat. No.
AccuPower® AHPND 1st PCR Kit, 0.2 ml thin-wall 8-tube strips with attached cap / 96 tubes *AccuPower*® AHPND 2nd PCR Kit, 0.2 ml thin-wall 8-tube strips with attached cap / 96 tubes	K-2923

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

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Preparation of 1st reaction mixture</td><td>1. After preparing the template DNA and nuclease-free water, add the template DNA to the AccuPower® AHPND 1st PCR Kit.

2. Add nuclease-free water into PCR tubes to make a total volume of 20 µl.
(Do not include the volume of the dried premix in the PCR tubes.)

3. Completely dissolve the vacuum-dried pellet by vortexing, and briefly spin down.</td></tr>
<tr><td>2</td><td> 
Incubate reactions in a thermal cycler</td><td>4. Place PCR tubes on the thermal cycler and perform the reaction under the following conditions.
<table><tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr><tr><td>Pre-denaturation</td><td>94°C</td><td>2 min</td><td>1 cycle</td></tr><tr><td>Denaturation</td><td>94°C</td><td>30 sec</td><td></td></tr><tr><td>Annealing</td><td>55°C</td><td>30 sec</td><td>39 cycles</td></tr><tr><td>Extension</td><td>72°C</td><td>90 sec</td><td></td></tr><tr><td>Final extension</td><td>72°C</td><td>2 min</td><td>1 cycle</td></tr></table>* Note: Users can adjust the protocol according to their instrument and template sequences to get optimal results.</td></tr>
<tr><td>3</td><td> 
Preparation of 2nd reaction mixture</td><td>5. After the 1st PCR reaction is completed, add 1-5 ul of the reaction solution into the AccuPower® AHPND 2nd PCR Kit.

6. Add nuclease-free water into PCR tubes to make a total volume of 20 µl.
(Do not include the volume of the dried premix in the PCR tubes.)

7. Completely dissolve the vacuum-dried pellet by vortexing, and briefly spin down.</td></tr>
<tr><td>4</td><td> 
Incubate reactions in a thermal cycler</td><td>8. After place PCR tubes on the thermal cycler, perform the reaction under the following conditions.
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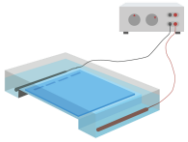
<tr><td>5</td><td> 
Analyze with gel electrophoresis</td><td>9. After the reaction, maintain the reaction mixture at 4-8°C.

10. Load samples on agarose gel without adding a loading-dye mixture, and perform gel electrophoresis for analysis.</td></tr>
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