V.3/Jan/2018

CODE No. GPQ320-100 100 tests

GenePasQ® Shrimp Pathogen Detection Kit

Multiplex & PAS

-AHPND/EMS, IHHNV, WSSV, EHP-

1. Introduction

For shrimp farming, it is very important to watch the health conditions of shrimp continuously. Particularly, the shrimp farmer is threatened with major diseases, namely; **EMS / AHPND** (*Vibrio parahaemolyticus*), **IHHNV** (Infectious Hypodermal and Haematopoietic Necrosis Virus, Type1, 2), **WSSV** (White Spot Syndrome Virus) and **EHP** (*Enterocytozoon hepatopenaei*). Using the conventional method, these are checked individually which take a long time and need special techniques. "**GenePasQ® Shrimp Pathogen Detection Kit**" was developed to detect these 4 diseases **simultaneously.** After PCR, you can **visually judge** the **existence** of the target genes with the ***PAS*** / DNA Chromatography method.

****PAS***: Printed Array-Strip (Patent licensed by NGK INSULATORS, Ltd.)

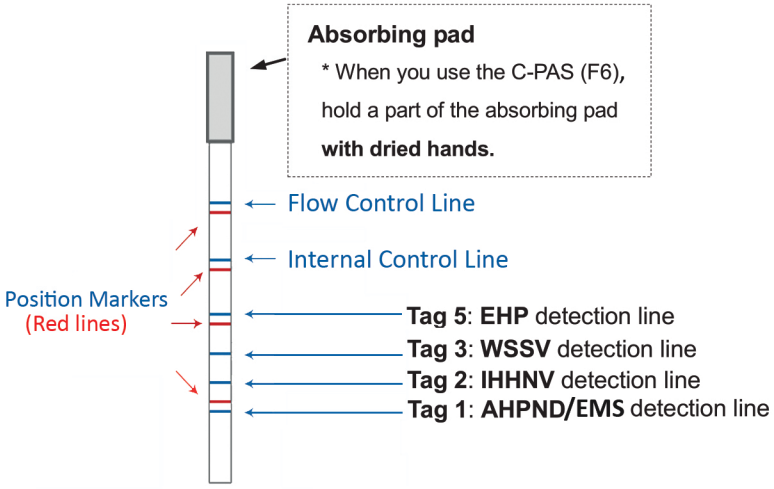
2. Characteristics

- Detect 4 diseases **simultaneously.**
  - **EHP** (*Enterocytozoon hepatopenaei*)
  - **WSSV** (White Spot Syndrome Virus)
  - **IHHNV** (Infectious Hypodermal and Haematopoietic Necrosis Virus)
  - **EMS / AHPND** (*Vibrio parahaemolyticus*)
- Electrophoresis is **not needed.**
- Easy to Use, Rapid & Safe
- Judge the results **by eyes** (visually).
- 10 times more sensitive than Gel Electrophoresis

3. Kit Contents (Good for 100 tests)

Label	Name of Component	Contents	Quantity
A	C-PAS(F6)	100 strips / vial	1 vial
	* Be sure to hold a part of absorbing pad of C-PAS(F6) **with dried hands** during use.		
B	Primer solution	240 µL / vial	1 vial
C	Latex solution	180 µL / vial	1 vial
D	Dilution buffer	1.2 mL / vial	2 vials

3-1. Explanation of C-PAS(F6)



- **Absorbing pad**

  Be sure to hold a part of the absorbing pad of C-PAS(F6) **with dried hands** during use.
- **Position markers (Red lines)**

  The position of emerging lines is referenced against these Position Markers (**Red lines**). These Position Markers always appear with or without reaction.
- **Flow control line**

  The (**blue**) line disappears when the solution passes through it.
- **Detection line**

  A (**blue**) positive line appears at the detection line if the target genes exist in your sample.

4. Equipment and Reagents necessary for the Test

<Not included in the Kit>

4-1 Reagents

- Premix PCR Enzymes

  We recommend the following reagents:

  SYBR Premix ExTaq (Tli RNaseH Plus) (Takara)

  Multiplex PCR Plus Kit (Qiagen: 206152 or Qiagen: 206151)
- RNase- Free Water

4-2 Instruments

- Gene amplification machine

We recommend the following units:

- **Quick Bath QB-0224B** (ThermoGen) — sold under **GenePasQ®** trademark
- GeneAmp 9700 (ABi)
- Mastercycler (Eppendorf)
- Veriti 200 (ABi)
- Rotor-Gene (Qiagen)

4-3 Other Instruments

- Vortex mixer (*BioSan* Multi Vortex V-32)
- Micropipettes (*METTLER TOLEDO* Rainin or Eppendorf)
- Homogenizer / Disruption Systems (*TOMY* Micro Smash MS-100 Beads Cell Disrupter)
- Thermo-Shaker (*BioSan* TS-100)
- Microcentrifuge (*BioSan* Microspin 12 and *TOMY* Multi Spin)

5. Protocol

5-1. Preparation of DNA solution

Please extract DNA from samples using your **predetermined** method.

We recommend the following product:

DNeasy Blood & Tissue kit (Qiagen: 69504)

5-2. Gene amplification by PCR

1) Prepare reaction mixture.

Add the following premix PCR solution to PCR tubes.

This kit is optimized for 10 µL of PCR system.

Premix PCR Enzyme	5.0 µL
Primer solution	2.0 µL
RNase- Free Water	2.0 µL
Total volume	9.0 µL

2) Add 1.0 µL of the sample DNA solution (Total 10 µL).

3) Amplify the nucleic acids by PCR.

Be sure to **close the PCR caps** to avoid liquid leakage and evaporation during PCR. Then, perform PCR under the following conditions.

When you use a gene amplification machine and PCR enzyme **other than our recommendation**, set the conditions of PCR based on the following conditions. Modification of the amplification conditions may be needed depending on the PCR machine and enzyme.

PCR conditions:

SYBR Premix ExTaq		
95℃	2 min	
95℃	15 sec	
60℃	15 sec	36
72℃	15 sec	cycles
4℃		

Multiplex PCR Plus Kit		
95℃	5 min	
95℃	15 sec	
60℃	30 sec	35
72℃	15 sec	cycles
4℃		

5-3. Reaction using the DNA Chromatography Method

1) Take out the "C) Latex solution" and "D) Dilution buffer" from the refrigerator and keep at room temperature.
2) Prepare the Latex-working solution by diluting "C) Latex solution" **1.5 : 20** in D) Dilution buffer". Mix the solution uniformly with a vortex mixer.
3) Centrifuge the PCR tubes after PCR briefly. Then, open the caps of PCR tubes after PCR and add 21.5μL of the Latex working solution. Mix the solution uniformly by pipetting.
4) Insert C-PAS(F6) in tube of step "3)". The **opposite end** of the absorbing pad must be inserted in the tubes.
※ Be sure to hold a part of the absorbing pad of C-PAS(F6) **with dried hands** during use. Touching the parts **other than the absorbing pad** and holding it with wet hands may result in an insufficient reaction.
5) Leave them at room temp. for 10 minutes for developing. Perform the reaction at room temp. (**20-30**℃) and **40-80%** Relative Humidity.

←Hold the C-PAS(F6) strip in this position **with dried hands** during use.

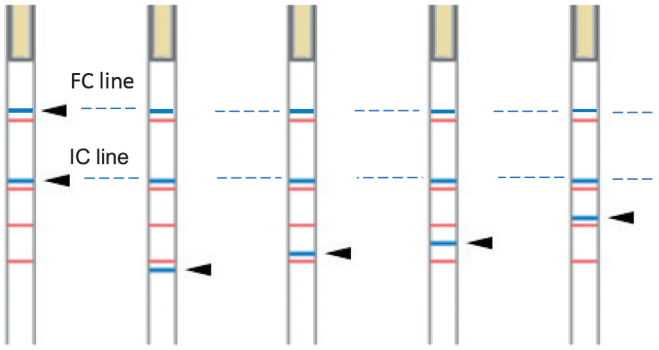
Caution: A low temp. and low humidity will cause **False-positive** detections.

5-5. Judgement of the Results

Negative: When no target gene exists in sample, the **blue** line at the **Internal Control** line appears.

Positive: When the target gene exists in the sample, the **blue** line at the detection line appears.

Detection lines	Targets
Tag 5	*Enterocytozoon hepatopenaei* (**EHP**)
Tag 3	White Spot Syndrome Virus (**WSSV**)
Tag 2	Infectious Hypodermal and Haematopoietic Necrosis Virus (**IHHNV**) ※If the shrimp genome contains the **IHHNV** gene fragment, it will test **positive** even if it is **not infected** with the **IHHNV**. Reference: Saksmerprome V., Jitrakorn S., Chayaburakul K., Laiphrom S., Boonsua K. & Flegel T. W. (2011) Additional random, single to multiple genome fragments of *Penaeus stylirostris* densovirus in the giant tiger shrimp genome have implications for viral disease diagnosis. Virus Research 160, 180-190.
Tag 1	*Vibrio parahaemolyticus* (**AHPND / EMS**)



Healthy Shrimp | **AHPND** (+) /**EMS** | **IHHNV** (+) | **WSSV** (+) | **EHP** (+)

* The **IC** (Internal Control) line often becomes faint when a target gene is amplified.

6. Storage Conditions and Expiry of the Kit

1) Store the kit at **2-8**℃ but **DO NOT** FREEZE.
2) Use the kit at 20-30℃. **After using it,** the kit must be returned to storage **at 2-8**℃ as soon as possible.
3) Do not use the kit after the expiration date indicated on the outside box.
4) The **DNA Positive Controls** must always be stored at **-20°C.**

WARRANTY

Nikken Bio Medical Laboratory, Inc. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made of are of standard quality. Buyer assumes all risks and liabilities resulting from the use of this product. There is no warranty of merchantability of the product or of its fitness for any purpose. Nikken Bio Medical Laboratory, Inc. agrees to replace any defective product but expressly disclaims any liability for damages including special or consequential damages or expenses arising directly or indirectly from the use of this product.

*** This product uses a DNA chromatography strip: C-PAS(F6), which is produced by TBA, Co., Ltd. with the Patent licensed by NGK INSULATORS, Ltd..**

- Exclusive Distributor: **SURE** Marketing Company, Inc. / **PT. SURE** Marketing Company
  Suite 706 Metropolitan Terraces Condominium
  Sacred Heart Street, San Antonio Village
  Makati City 1203, **PHILIPPINES**
  Phone: +63 2 897-24-30 up to 31
  E-Mail: smci@sure-bio.com
  URL: www.sure-bio.com
- In collaboration with **TBA Co. , Ltd. (C-PAS Supplier)**
- **Manufacturer: Nikken Bio Medical Laboratory, Inc.**
- Product development done by **Tokyo University of Marine Science and Technology (TUMSAT)** under **Prof. Ikuo Hirono**

GPQPI - STD vers 03 - 07 Jan 2018