

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*), **IHNV** (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.)

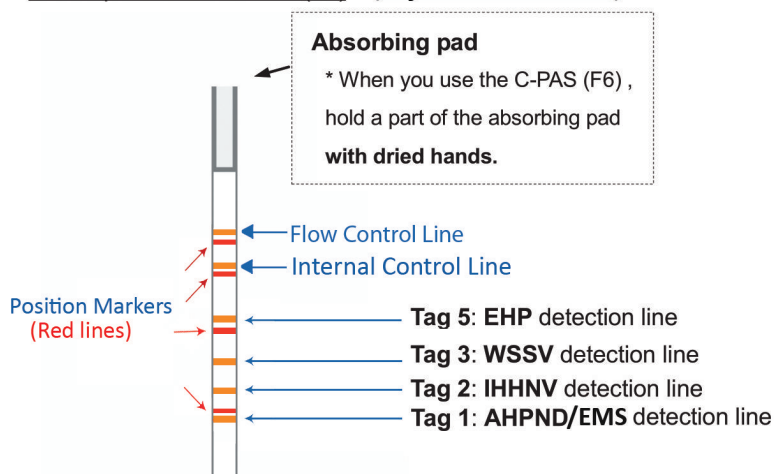
2. Characteristics

- ◆ Detect 4 diseases **simultaneously**.
 - **EHP** (*Enterocytozoon hepatopenaei*)
 - **WSSV** (White Spot Syndrome Virus)
 - **IHNV** (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 50 tests)

Label	Name of Component	Contents	Quantity
A	C-PAS(F6)	50 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	120 µL / vial	1 vial
C	Latex solution	90 µL / vial	1 vial
D	Dilution buffer	1.2 mL / vial	1 vial

3-1. Explanation of C-PAS(F6) **(Before the reaction)**



• 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 **orange** 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)
- 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)
- GenePasQ[®] C-PAS Image Reader

5. Protocol

5-1. Preparation of DNA solution

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

We recommend the following products:

- **GenCheck Extraction Reagent**
- DNeasy Blood & Tissue Kit (Qiagen: 69504)

5-2. Gene amplification by PCR

1) Prepare reaction mixture. **(Place them on ice.)**

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 or Positive Control (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**, modification of the amplification conditions may be needed depending on the PCR machine and enzyme. Set the conditions of PCR based on the following conditions:

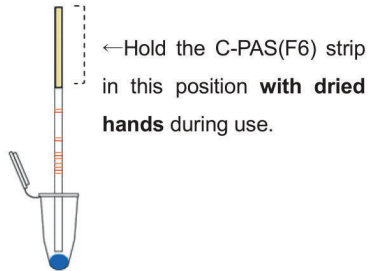
PCR conditions:

SYBR Premix ExTaq		Multiplex PCR Plus Kit	
95°C	2 min	95°C	5 min
95°C	15 sec	95°C	15 sec
60°C	15 sec	60°C	30 sec
72°C	15 sec	72°C	15 sec
4°C		4°C	
36 cycles		35 cycles	

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°C) and 40-80% Relative Humidity.**



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

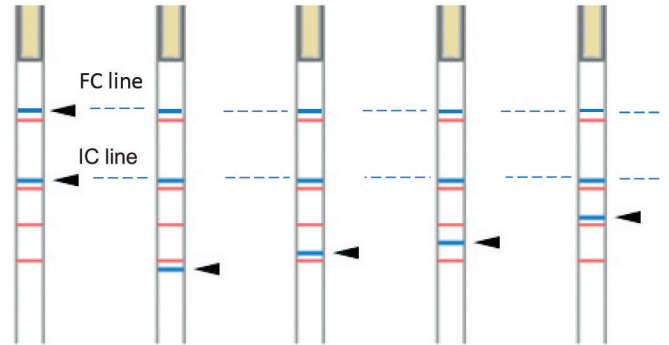
5-5. Judgement of the Results

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 line	Target
Tag 5	<i>Enterocytozoon hepatopenaei</i> (EHP)
Tag 3	White Spot Syndrome Virus (WSSV)
Tag 2	Infectious Hypodermal and Haematopoietic Necrosis Virus (IHNV) ※If the shrimp genome contains the IHNV gene fragment, it will test positive even if it is not infected with the IHNV. Reference: Saksmerprom V., Jitrakorn S., Chayaburakul K., Laiphrom S., Boonsua K. & Flegel T. W. (2011) Additional random, single to multiple genome fragments of <i>Penaeus stylirostris</i> densovirus in

	the giant tiger shrimp genome have implications for viral disease diagnosis. Virus Research 160, 180-190.
Tag 1	<i>Vibrio parahaemolyticus</i> (AHPND / EMS)



Healthy Shrimp **AHPND (+)** **IHNV (+)** **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°C** but **DO NOT FREEZE.**
- 2) Use the kit at 20-30°C. **After using it,** the kit must be returned to storage **at 2-8°C** 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.**

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This GenePasQ® Kit is produced by TBA, Co., Ltd. and uses DNA Chromatography Strips: C-PAS (F6) with a Patent licensed from NGK Insulators, Ltd.

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- The primers were developed by Tokyo University of Marine Science and Technology (TUMSAT) under Prof. Ikuo Hirono.