Quick Guide Protocol

- Protocol of GenePasQ® DNA 1, GenePasQ® DNA 2, & GenePasQ® DNA 3

1) Preparation of the DNA solution

← Extract DNA from samples with recommended reagent and equipment.

Please use the proper DNA extraction method as shown below.

← Obtain SAMPLE [For Animal (Shrimp) sample, use **less than 10mg**]
HOMOGENIZE manually.

← Add **40µl** of the **GenCheck** or **any Recommended** extraction reagent.

← Vortex.

← Centrifuge at **10000 rpm for 30 seconds.**

← Heat block (Thermo-Shaker) at 98-100 °C for 10 minutes.

← Stay on ice for 1 minute.

← Centrifuge at **10000 rpm for 5 minutes.**

← Transfer **5µl** of the **Supernatant liquid** to a **new** 1.5ml tube.

← Add **95µl** of the **RNase-free water.**

Recommended Equipment:

- PCR machine — Quick Bath (ThermoGen) - Sold under the GenePasQ® trademark.
  GeneAmp 9700 (ABi), Veriti 200 (ABi), Mastercycler (Eppendorf)
  Rotor-Gene (Qiagen)

2) Preparation of Reaction Mixture

You should prepare enough volume of working solution
9µL x number of tests (samples)

Need 9µL / test			
*1) Premix PCR Enzyme	5.0 µL	µL	☐
B) Primer solution	2.0 µL	µL	☐
*2) RNase-Free Water	2.0 µL	µL	☐
Total volume	9.0 µL	µL	☐

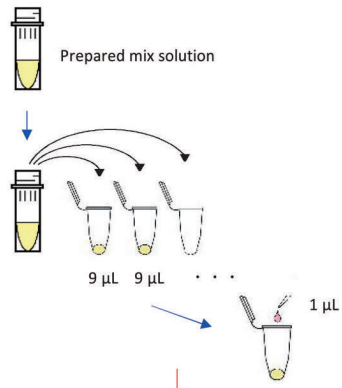
*1) Premix PCR Enzyme : Recommended

① **Multiplex PCR Plus Kit (Qiagen: 206152)**

② **SYBR Premix ExTaq**

*2) RNase-Free Water : Please prepare individually.

← Dispense the premix PCR solution to each PCR tube.



3) Addition of the sample DNA solution

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

4) Amplify the DNA by PCR

	SYBR Premix ExTaq			Multiplex PCR Plus Kit			(Other Protocol)		
①	95℃	2 min		95℃	5 min		℃	min	
②	95℃	15 sec		95℃	15 sec		℃	sec	
③	60℃	15 sec	36 cycles	60℃	30 sec	35 cycles	℃	sec	cycles
④	72℃	15 sec		72℃	15 sec		℃	sec	
⑤	4℃			4℃			℃		

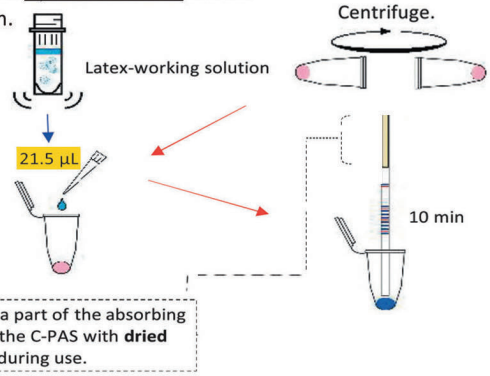
5) Prepare the Latex-working solution during the PCR step.

← C) Latex solution : D) Dilution buffer = 1.5 : 20

	need 21.5µL / test	x number of tests	
C) Latex solution	1.5 µL	µL	☐
D) Dilution buffer	20.0 µL	µL	☐
Total volume	21.5 µL	µL	☐

← Mix the solution uniformly with vortex mixer. (Latex-working solution)

* Keep at room temperature the C)Latex solution and D)Dilution buffer before using them.



* Hold a part of the absorbing pad of the C-PAS with **dried hands** during use.

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6) Reaction using the C-PAS

← Perform the following Steps in a STERILE room.
← Centrifuge the PCR tubes after PCR briefly.
← Add 21.5 µL of the Latex-working solution.
← Mix the solution uniformly by pipetting.
← Insert the C-PAS in tube.
← Leave them at room temperature for 10 minutes.
* Perform the reaction at room temperature (20-30°C) and 40-80% humidity.

CAUTION : A **low temperature** and **low humidity** will cause **FALSE-positive** detections.

7) Identification of the Shrimp Pathogen (s)

Read the PAS Line with the aid of the **GenePasQ®** Data Sheet / Strip Reading Guide.

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.

• Exclusive Distributor: PT. SURE Marketing Company

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