- Protocol of GenePasQ®-

CODE No. GPQ320-100 / GPQ 350-100 / GPQ 370-100

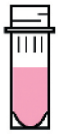
1) Preparation of the DNA Solution

← Extract DNA from samples with the recommended reagent and equipment.

Please use the proper DNA Extraction Method.

Recommended Reagent and Equipment:

- Reagent **DNeasy Blood & Tissue Kit (Qiagen: 69504)**
- PCR Machine Quick Bath (ThermoGen) - Sold under the GenePasQ® trademark. GeneAmp 9700 (ABi), Veriti 200 (ABi), Mastercycler (Eppendorf), Rotor-Gene (Qiagen)



Extracted DNA

2) Preparation of Reaction Mixture

You should prepare enough volume of the working solution.

	Need 9µL / test	9µL x number of tests (samples)
*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 ☐

Prepared mix solution

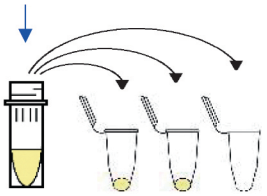
*1) Premix PCR Enzyme : Recommended

① **Multiplex PCR Plus Kit (Qiagen: 206152** or **Qiagen No. 206151)**

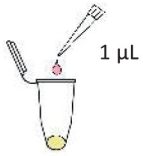
② **SYBR Premix ExTaq**

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

← Dispense the premix PCR solution to each PCR tube.



9 µL 9 µL · · ·



1 µL

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.

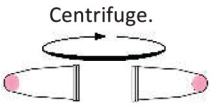
← C) Latex solution : D) Dilution buffer = 1.5 : 20

	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 ☐

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

Latex-working solution

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



Centrifuge.

21.5 µL

6) Reaction using the C-PAS Strip

← Centrifuge the PCR tubes after PCR briefly.

← Add 21.5 µL of the Latex-working solution.

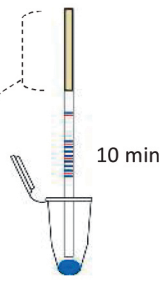
← Mix the solution uniformly by pipetting.

← Insert the C-PAS strips inside the PCR tubes.

← Leave them at room temperature for 10 minutes.

* Perform the reaction at room temperature (20-30°C) and 40-80% humidity.

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



10 min

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

7) Read the PAS Line

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

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