IQ Plus™ Extraction Kit

DNA/RNA Co-extraction for IQ Plus™ System

User Manual

***in vitro* use only**

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I. Intended use

IQ Plus™ Extraction Kit is intended to extract DNA and RNA from shrimp specimen simultaneously to be used for **IQ Plus™** detection kits.

The intended user of the **IQ Plus™** Extraction Kit shall be the technician who works in shrimp farm or hatchery.

This kit is for *in vitro* use only.

II. Introduction

IQ Plus™ Extraction Kit is designed to generate DNA or RNA extract served as the template for **IQ Plus™** detection kits. By using this kit, the DNA and RNA can be extracted simultaneously from various types of penaeid shrimp tissues.

IQ Plus™ Extraction Kit is using a spin column based DNA/RNA co-extraction system. The users can collect 10-100 ng/µl nucleic acid in 10 minutes.

III. Product Description

Material provided

IQ Plus™ Extraction Kit (50 tests/kit)

Item	Volume	Note: Before first use,
Solution 1	26 ml/bottle	
Solution 2	12 ml/bottle	Add 48 ml 95% ethanol before use
Solution 3	20 ml/bottle	
Spin column & Collection tube	50 sets/pack	
Grinder	50 pcs/pack	

Materials and Equipments Required, but Not Provided

1. **cubee™** Mini-Centrifuge
2. Micropipette and tips
3. Micro-centrifuge tubes
4. 95% ethanol

IV. Storage

The kit should be stored at room temperature.

V. Precaution

1. Do not reuse the Spin Column & Collection Tube.
2. We strongly recommend that the working area for extraction procedure and amplification procedure should be separated in two independent spaces to avoid any possible contamination.
3. We strongly recommend to use aerosol free tips to avoid any possible contamination.
4. Always wear gloves when using the kit to avoid the irritation caused by the extraction solution.
5. Please shake the bottle before use to make sure the solution is homogenized.
6. In order to avoid the evaporation of the solution during storage, please cap the bottle tightly after each use.
7. Any deviation from the instruction may lead to a low recovery rate.

VI. Sample Type

1. The target tissues can be one of the following:

Sample Type	Sample Size / Sample Pretreatment	
Post larvae (PL)	< PL6 : 25 - 50 PLs	
	PL6 - PL15 : 10 - 30 PLs	
Fantail	Shrimp 1 - 2cm : 10 pieces	
	Shrimp 2 - 4cm : 5 pieces	
Pleopod	Shrimp 4 - 7cm : 10 pieces	
	Shrimp 7 - 10cm : 3 pieces	
	Shrimp 10 - 15cm : 1 piece	
	Shrimp 15 - 20cm : 1/3 pleopod	
Stomach	1 piece	
Mid-gut	1 cm	
Hepatopancreas	Fresh	For 15 to 20 g size shrimp: about 10%
	Ethanol preserved	For 15 to 20 g size shrimp: about 25%
Fecal sample	1 cm	
Pond water	1.5 ml pond water; first centrifuge at 13,000 rpm or by **cubee™** Mini-Centrifuge for 3 minutes. Discard the supernatant, then resuspend the pellet with 100 µl PBS.	

NOTE: The sample size from the different sample sources and different size of shrimp should be adjusted accordingly. For example, more hepatopancreas should be used if shrimp size is smaller than 15 to 20 g.

2. Sampling instructions:

a) Beside hepatopancreas specimen, the sample can be

fresh, ethanol-preserved or frozen shrimp tissues.

b) Hepatopancreas specimen should be fresh or ethanol-preserved.

c) If the sample needs to be ethanol-preserved, please follow the recommendation as below: Preserve the tissue in 95% ethanol at room temperature. The volume of the ethanol should be twice of the tissue.

VII. Operation Procedure

1. Place sample into a clean 1.5 ml microcentrifuge tube with 500 µl Solution 1.
   - **NOTE: For pond water sample, please skip step 2.**
2. Use a grinder to homogenize sample inside the tube.
3. Add 500 µl Solution 2 (with ethanol) into the tube.
4. Mix thoroughly and spin for 1 minute.
5. Transfer 500 µl supernatant to the spin column & collection tube set.
6. Spin for 1 minute and discard the flow-through from the collection tube. Reassemble the collection tube to the spin column.
7. Add 500 µl Solution 2 into the spin column & collection tube set.
8. Spin for 3 minutes and discard the flow-though.
9. Transfer the spin column to a clean 1.5 ml microcentrifuge tube.
10. Add 200 µl Solution 3 into the spin column.
    - **Note: For hepatopancreas sample, please add 400 µl Solution 3 into the spin column.**

- **Note: The volume of solution 3 is subject to change according to the sample type. Please refer to individual detection kit manual.**

11. Spin for 1 minute. The nucleic acid extract is eluted into the 1.5 ml microcentrifuge tube and is ready to use.

- **Note: Extracted nucleic acid for IMNV detection should be heated at 95℃ for 5 minutes then quench on ice immediately before use.**

Operation Procedure

- **Place sample into a clean 1.5 ml microcentrifuge tube with 500 µl Solution 1.**
- **Use a grinder to homogenize tissue sample.**

- **Add 500 µl Solution 2 (with ethanol).**

- **Mix thoroughly and spin for 1 minute.**

- **Transfer 500 µl supernatant sample to the spin column & collection tube set.**

- **Spin for 1 minute.**
- **Discard the flow-through.**

- **Add 500 µl Solution 2.**

- **Spin for 3 minutes.**
- **Discard the collection tube containing the filtrate.**

- **Place the spin column into a clean 1.5 ml microcentrifuge tube.**
- **Add 200 µl Solution 3.**

(For hepatopancreas sample, please add 400 µl Solution 3.)

- **Spin for 1 minute.**
- **Discard the spin column. (Keep microcentrifuge tube containing the liquid sample.)**

Nucleic acid extract.

VIII. The Stability of Nucleic Acid Extract

1. The extracted nucleic acid can be stored at room temperature for up to one hour. However, we strongly recommend performing the downstream reaction procedure as soon as possible.
2. If the storage is necessary, the extracted nucleic acid can be stored for up to one month at -20°C.

IX. Troubleshooting

Problems	Possible causes	Comments or solution
Failure in downstream experiment	1) Incorrect preparation of the reagent.	■ Check to ensure the solution preparation procedure is correct. ■ Check to ensure the correct volume of 95% ethanol is added to Solution 2.
	2) Deterioration of the reagents.	■ Check the expiration date and storage condition.
	3) Vaporization of Ethanol in Solution 2 during storage.	■ Close the cap tightly after each use.
	4) Poor nucleic acid quality.	■ Please confirm the sample storage condition. ■ Do not add too much sample. Please follow the recommendation in this User Manual.
	5) PCR inhibitor.	■ Do not add too much sample. Please follow the recommendation of this user manual. ■ Spike nucleic acid sample into P(+) Control reaction for a parallel PCR reaction. Negative results indicate the presence of inhibitors in the nucleic acids. In that case, prepare another nucleic acid extract.
No flow through	1) Tissue stock on the column.	■ Please carefully transfer the supernatant from step 5, and avoid adding the tissue into the column.
	2) Centrifuge force is not enough.	■ Please use cubee or high speed centrifuge to spin.
Precipitation in Solution 1	1) Storage temperature is too low.	■ Put the bottle in a 40±5℃ water bath for 10 min.

X. References

1. Chou, P. H., Lin, Y. C., Teng, P. H., Chen, C. L., Lee, P. Y. (2011). Real-time target-specific detection of loop-mediated isothermal amplification for white spot virus using fluorescence energy transfer-based probes. *Journal of Virological Methods* 173 (1): 67-74.

2. Duangsuwan, P., Tinikul, Y., Chotwiwatthanakun, C., Vanichviriyakit, R., Sobhon, P., (2008) Changes in the histological organization and spheroid formation in lymphoid organ of *Penaeus monodon* infected with yellow head virus. *Fish & Shellfish Immunology* 25 (5):560-569.