

Aquatic Animal Pathogen Genomic DNA/RNA Extraction Kit (FAST)

Introduction

Aquatic Animal Pathogen Genomic DNA/RNA Extraction Kit (FAST) is suitable for quickly extracting high-purity pathogenic genomic DNA and RNA from animal tissue homogenates. This kit is based on silica gel column purification technology. There is no need to use toxic phenol chloroform extraction during the extraction process, nor time-consuming alcohol precipitation. The obtained nucleic acid can be directly used for downstream experiments such as PCR/RT-PCR, Southern hybridization/Northern hybridization, LAMP/RT-LAMP.

Principle

Animal total DNA/RNA extraction kit is based on the silica gel column purification method. The nucleic acid is released under the action of a lysate. When the nucleic acid passes through the silica gel column in a high salt environment, it will be adsorbed on the membrane of the silica gel column, and the protein is not adsorbed. The nucleic acid-adsorbing filter is washed to remove residual proteins and salts. Finally, DEPC can be used to treat the nucleic acid adsorbed on the water-eluting filter. The obtained nucleic acid has high purity and can be directly used in various downstream experiments.

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Product	Content
Spin column (casing)	48
Solution 1	25 mL
Solution 2	60 mL
Solution 3	10 mL
Manual	1

Contents

Storage and Stability

This product can be stored at room temperature (15-25°C) for 12 months. Long-term storage should be placed in 2-8°C.

Attention

- 1. To ensure the accuracy of test results, please follow the instructions strictly.
- 2. Spin column in the kit are disposable products. Please do not reuse them.
- 3. All waste products used for testing should be placed in a waste tank containing disinfectant to be soaked and disinfected; after the experiment, disinfect the table with 1% sodium hypochlorite or 75% alcohol immediately.
- 4. During the experiment, the experimenters should wear gloves, masks and lab coats to avoid contacting with reagents and samples.
- 5. During the experiment, try to avoid contacting with the nozzle of Spin column and centrifugal tube. If any sample solution sticks to the gloves or splashes during the operation, change the gloves immediately and clean the splashes. All articles that come into contact with diseased materials should be disposed properly.
- **6.** This product is only for the use of aquatic animal pathogen detection experiments, do not use for other purposes.



Materials and Equipment required

- Sterile saline
- Clean tweezers and scissors
- Homogenizer, homogenizing bag or disposable grinding rod
- Micropipettor (100-1000 µl, 10-100 µl)
- DNase/RNase-free sterile centrifuge tube (1.5 mL or 2 mL)
- DNase/RNase-free sterile tip
- Vortex mixer
- Centrifuge (Speed ≥ 10,000 rpm)

Procedure

1. Sample Preparation (Pre-treatment):

(1) Shrimp seed, fish fry, animal tissue (such as Shrimp tissue, fish tissue, artemia larvae, mollusk tissue, etc.) samples: take 3~5 g of the sample into a homogeneous bag, add 3 times the volume of normal saline ,and grind the samples thoroughly.

(2) Clamworm: Take 5-10 samples of clamworms, cut $2\sim3$ cm from each caterpillar, put them in a homogenization bag, add 3 times the volume of normal saline, and grind the samples thoroughly.

(3) Feed samples: Take 2~4 g of the sample into a homogenization bag, add 4 times the volume of normal saline, and grind the samples thoroughly.

(4) Aquaculture water sample: First filter impurities with filter paper, and then filter with a $0.22 \ \mu m$ water filter membrane (aqueous filter membrane is not easy to block) (the filter membrane can retain bacterial pathogens such as EHP, Vibrio, etc.), more than 1 L of culture water needs to be filtered. Cut the filter membrane into small pieces and put them into a centrifuge tube, add physiological saline to cover the filter membrane, vortex and shake thoroughly.

2.Take the 200 μ l sample prepared in step 1 into a 1.5 mL centrifuge tube, add 400 μ l of solution 1 to the centrifuge tube, shake and mix for 30 seconds. Leave it at room temperature for 5-10 minutes and centrifuge it at 10,000 rpm for 3 minutes to remove precipitate.

3. Take out the spin column (casing), transfer as much of the supernatant as possible from the previous step to the column (be careful not to suck the precipitate), and centrifuge it at 10,000 rpm for 1 minute.

4. After discarding the filtrate, put the column back into the collection tube, add 500 μ L of solution 2 to the column, and centrifuge it at 10,000 rpm for 1 minute.

5. Repeat step 4 once.

6. After discarding the filtrate, put the column back into the collection tube, centrifuge it at 10,000 rpm for 2 minutes to dry the column.

7. Transfer the column to a new 1.5 mL centrifuge tube and add 100 μ L of solution 3 to the center of the column membrane. Leave it at room temperature for 1 minute and centrifuge it at 10,000 rpm for 1 minute. Store DNA/RNA at -20°C.

Manufacturer

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