GENOMIC DNA EXTRACTION KIT

-Intended for purification of DNA from shrimp samples

CATALOG NUMBER: DIG-EX-25

For Research Use Only

Contents

Kit Contents for 25 reactions

Reagents	Volume/ No.of bottle
Lysis buffer	15 ml × 1 bottle
Neutralization or binding buffer	10 ml × 1 bottle
Wash buffer I	2 ml × 1 bottle
Wash buffer II	6 ml × 1 bottle
TE buffer	1.5 ml × 1 vial
Spin columns	25 numbers

Storage:

The DNA extraction kit should be stored dry at room temperature (15–25°C) and are stable for a year under these conditions. Keep the solution in a tightly-closed container. Store in a dry, cool and well-ventilated place. Do not refrigerate, as some of the components may precipitate out of solution.

Safety Information

Personal precautions, protective equipment and emergency procedures:
Ensure adequate ventilation. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
General information:
This mixture is essentially salty detergent solution which is used in many household cleaning and hygiene products. The solution supplied is less concentrated than one might encounter in the home. The principal hazards from this mixture are skin and eye contact.
Inhalation: Move the casualty to fresh air. If respiratory problems occur, consult a doctor.
Skin contact: Remove contaminated clothing, which can then be washed as normal. Wash the liquid off the skin immediately with plenty of water. Seek medical attention if irritation occurs.
Eye contact: Rinse opened eye immediately with running water, also wash under the eyelids, for several minutes. Seek medical advice if irritation persists.
Ingestion: Rinse out mouth with water, then drink plenty of water. Do not induce vomiting.
Self-protection of the first aider: Rinse your hands with water after handling anything that has been contaminated with the solution.
Most important symptoms and effects, both acute and delayed vision-Irritation to the eyes (burning sensation, redness and impairment of-- similar to getting soap in the eye).
Indication of any immediate medical attention and special treatment: First Aid as outlined above, decontamination of clothing etc, treatment a medical professional if symptoms persist.

CAUTION: Do not add bleach or acidic solutions directly to the sample-preparation waste as buffer contains highly reactive compounds. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Introduction:

The DNA extraction kit is intended for rapid extraction of genomic DNA from samples including tissues using spin column technology. The steps involved in this kit includes lysis, binding, washing, and elution. Silica-based technologies allows DNA adsorption specifically to silica membranes in the presence of certain salts and at a defined pH. Initially the target

tissues are lysed to release nucleic acid, followed by binding of nucleic acid to a silica membrane and washing away particulates, inhibitors that are not bound to the silica membrane, and elution of the nucleic acid in a low salt buffer or elution buffer. Chaotropic salts are included in the kit buffers to aid in protein denaturation and extraction of DNA. This method is incorporated in spin columns which is cost-effective, has a simpler and faster procedure than the organic extraction.

Things to do before starting

- ✓ Wash Buffer 1 and Buffer 2 are supplied as concentrates. Before using for the first time, reconstitute the wash buffers 1 and 2 with 100 % ethanol as indicated on the bottle to obtain a working solution.
- ✓ All centrifugation steps are carried out at room temperature (15–25°C).
- ✓ Prepare 95 °C water bath for use in step 5.

Type of samples

This kit is intended for testing live, frozen, and ethanol-preserved samples

- **Broodstock -** Faecal sample & pleopods
- **Hatcheries-** Post larvae
- **Farms-**Apparently healthy animals at juvenile/adult stage

For WSSV detection, the target organs includes

- **Post Larvae** -15-20 pieces
- **Pleopods**- 4 pieces

For EHP detection, the target organs includes

- **Hepatopancreas**-20-100 mg

Equipment and reagents to be supplied by User

- Micro pestle homogeniser
- Dry bath - 95 °C
- Centrifuge
- Nano spectrophotometer
- Pipettes and filter tips
- Micro centrifuge tubes
- Ethanol-100 %
- Isopropanol-100 %

Protocol

1. Initially the collected samples are cut into small pieces using sterile scissors.
2. 20-100 mg of finely minced samples are transferred into micro centrifuge tube.
3. To the sample, 0.2 ml of lysis buffer is added followed by homogenisation.
4. The sample is then made up to 0.5 ml with lysis buffer.
5. The homogenised samples are incubated at 95 °C for 10 minutes.
6. After incubation, the samples are allowed to cool and then centrifuged at 8000 rpm for 5 minutes.
7. After centrifugation, 250 µl of the clear supernatant is collected into a fresh tube without disturbing the pellet.
8. To the collected supernatant, 350 µl of neutralisation or binding buffer is added and mixed thoroughly by inversion.
9. The samples are again centrifuged at 8000 rpm for 5 minutes.
10. To the 350 µl of the resulting supernatant, 350 µl of 100 % isopropanol is added and the tube is mixed thoroughly by inversion.
11. The above mixture is now applied to the spin column and centrifuged at 8000 rpm for 1 min.
12. The spin column is washed with 700 µl of wash buffer 1 and centrifuged at 8000 rpm for 1 min.
13. The spin column is again washed with 700 µl of wash buffer 2 and centrifuged at 8000 rpm for 1 min.
14. Finally the flow through is discarded and the column is centrifuged at 8000 rpm for 2 minutes to air dry the membrane completely.
15. DNA is eluted with 50 µl of nuclease free water or TE buffer and quantified using Nano spectrophotometer.

Determination of yield and purity

The ratio of absorbance at 260 and 280 nm is used to access DNA purity. Pure DNA has A_{260}/A_{280} ratio of 1.7–1.9. If the ratio is appreciably lower (≤ 1.6) it may indicate the presence of proteins, phenol or other contaminants that absorbs strongly at or near 280 nm.

Troubleshooting Guide

- Lower DNA yields
  - Starting sample size was insufficient
  - Improper storage of samples or repeated freezing.
  - Cells were not lysed thoroughly.
- Poor DNA yield
  - Low pH of some deionized water may reduce the DNA yield
  - Check the pH of the water before eluting the DNA (pH -7).
- DNA does not perform well in downstream applications
  - This includes ethanol carryover due to incomplete removal of ethanol by performing an empty spin and salt carryover due to improper dilutions of buffer solutions.
  - Be sure to thoroughly air dry the sample prior to elution.
- Low yield due to insufficient lysis
  - Make sure to homogenise the sample completely by forming complete suspension.
- Degraded DNA
  - Avoid carryover contamination and use filter tips while handling of sample during extraction under sterile condition.
  - Ensure proper storage of DNA and minimize the shearing of DNA during extraction and check the integrity of the DNA by running gel electrophoresis.
- Low DNA quality
  - $A_{260}/A_{280} < 1.7$: Proteins or chaotropes may be present-
    - Membrane is clogged with tissue fibers
    - Incomplete digestion of the tissue sample
  - $A_{260}/A_{280} > 1.9$*:* RNA may be present-
    - Too much input material
    - Lysis time is insufficient

TECHNICAL ASSISTANCE

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