

Marine Animals Tissue DNA Kit

Cat. No.: M1522830 | Pack size: 50T | Storage: Room temperature

Overview

This kit is suitable for rapid and simple extraction of genomic DNA from marine animal tissue samples. The extracted genomic DNA has large fragments, high purity, and good stability, and can be directly used in experiments such as PCR, restriction enzyme digestion, and hybridization. The extraction process does not require phenol-chloroform extraction. Blood, cells, or tissues are lysed with a lysis buffer and digested with proteinase K, then in the presence of absolute ethanol the DNA binds to the WHF spin column. After thorough washing to remove residual proteins, salts, and other impurities, the DNA is finally eluted with Buffer EB.

Kit Contents & Storage

Product No.	Component	Appearance	Size	Storage
M1522830A	Buffer UA	Liquid	15 mL	RT
M1522830B	Buffer UL	Liquid	15 mL	RT
M1522830C	Buffer PW1	Liquid	17 mL	RT
M1522830D	Buffer PW3	Liquid	15 mL	RT
M1522830E	Buffer EB	Liquid	15 mL	RT
M1522830F	Proteinase K	Liquid	1.2 mL	RT
M1522830G	WHF Spin Column (with 2 mL collection tube)	—	1 EA × 50	RT

All components of this kit can be stored under dry conditions at room temperature for 15 months.

Before use, check Buffer UA for crystallization or precipitation. If present, redissolve by placing in a 37°C water bath.

Precautions

1. Samples should be protected from repeated freeze-thaw cycles, which may result in smaller DNA fragments and reduced yield.
2. If RNA removal is required, the user needs to supply RNase A solution (100 mg/mL).
3. The user needs to supply absolute ethanol.

Procedure

Before use, add 22 mL of absolute ethanol to Buffer PW1 and 60 mL of absolute ethanol to Buffer PW3.

1. Sample Processing

Cut no more than 30 mg of marine animal tissue and place into a centrifuge tube containing 200 μ L of Buffer UA. Vortex for 15 sec.

Note: The starting amount varies slightly depending on the tissue type. Gill tissue contains a high cell mass; generally, it is recommended not to exceed 20 mg. If RNA removal is required, add 4 μ L of RNase A solution (100 mg/mL), vortex for 15 sec, and let stand at room temperature for 5–10 min.

2. Add 20 μ L of Proteinase K and vortex to mix. Incubate at 56°C until the tissue is completely dissolved. Briefly centrifuge to collect the solution from the cap wall, then proceed to the next step.

Note: The lysis time varies among different marine animal tissues, typically 0.5–2 h. Scallop tissue usually lyses completely within 0.5 h; shrimp and fish tissues take about 1 h. During incubation, mix the sample by vortexing for 15 sec 2–3 times per hour to promote tissue lysis.

3. Add 200 μ L of Buffer UL and mix thoroughly by inversion. Incubate at 70°C for 10 min. Briefly centrifuge to collect the solution from the cap wall.

Note: White precipitate may form upon addition of Buffer UL, but it usually disappears during incubation at 70°C and does not affect subsequent steps.

4. Add 200 μ L of absolute ethanol and mix thoroughly by inversion. Flocculent precipitate may appear (this is normal). Transfer the solution and precipitate together into a WHF spin column (placed in the provided 2 mL collection tube). Centrifuge at 12,000 rpm (13,680 $\times$ g) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

5. Add 700 μ L of Buffer PW1 (with ethanol pre-added) to the spin column. Centrifuge at 12,000 rpm (13,680 $\times$ g) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

6. Add 700 μ L of Buffer PW3 (with ethanol pre-added) to the spin column. Centrifuge at 12,000 rpm (13,680 $\times$ g) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

7. Repeat step 6 (second wash) to further remove residual impurities on the spin column and increase DNA purity.

8. Centrifuge the spin column together with the collection tube at 12,000 rpm (13,680 $\times$ g) for 2 min to completely remove residual wash buffer and impurities (this avoids residual ethanol affecting subsequent DNA elution and experiments).

9. DNA elution

Transfer the spin column to a new 1.5 mL centrifuge tube. Open the cap and let stand for 2 min to allow residual ethanol to evaporate completely. Slowly add 50–200 μ L of Buffer EB dropwise to the

center of the membrane without touching it. Let stand at room temperature for 1 min. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 sec. Collect the DNA solution in the centrifuge tube. The DNA can be used immediately for downstream experiments or stored at -20°C for long-term preservation.

Notes

- (1) If downstream applications are sensitive to pH or EDTA, sterile water can be used for elution instead. The pH of the elution buffer significantly affects elution efficiency. If water is used, ensure its pH is between 7.0 and 8.5 (adjust with NaOH). pH values below 7.0 will reduce elution efficiency.
- (2) To increase the final DNA concentration, reload the collected DNA eluate from step 9 onto the same membrane, let stand at room temperature for 2 min, centrifuge at 12,000 rpm ($13,680 \times g$) for 30 sec, and collect the eluate again.
- (3) For long-term DNA storage, it is recommended to elute with Buffer EB and store the DNA solution at -20°C to effectively prevent DNA degradation.

DNA Concentration and Purity Assessment

The fragment size of the extracted genomic DNA is influenced by factors such as sample storage time and shear forces during handling. The purified DNA can be assessed for concentration and purity by agarose gel electrophoresis and UV spectrophotometry.

1. Concentration determination

Measure the absorbance of the sample at 260 nm (A_{260}) using a UV spectrophotometer to determine DNA concentration. If dilution is required, Buffer EB is recommended. Dilution with water may lead to inaccurate readings. The absorbance at 260 nm should be kept within 0.1–1.0 to ensure accuracy. Conversion: 1 unit of absorbance at 260 nm corresponds to 50 $\mu\text{g}/\text{mL}$ of double-stranded DNA or 40 $\mu\text{g}/\text{mL}$ of single-stranded DNA.

2. Purity assessment

Evaluate DNA purity by the A_{260}/A_{280} ratio. For high-quality genomic DNA, the A_{260}/A_{280} ratio should be between 1.7 and 1.9. If ddH₂O instead of Buffer EB is used for elution, the ratio may be lower due to the influence of pH and ions on light absorption; this does not necessarily indicate low DNA purity.

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Aladdin Scientific Corporation

14078 Meridian Parkway, Riverside, CA 92518, USA

Phone: 1-833-552-7181

Sales: sales@aladdinsci.com

Customer Service: custserv@aladdinsci.com

EU SALES, LOGISTICS & LOCALIZED SUPPORT

Aladdin Biochem Deutschland GmbH

Westring 2, 33142 Büren, Germany

Phone: +02951 9383958

Support: support.eu@aladdinsci.com

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