Genomic DNA Isolation from 5–10 mg Marine Invertebrate Tissue

AquaPure® Genomic DNA Isolation Kit

Catalog #732-6340
Expected yield range: 2–10 µg DNA

Method

Cell Lysis

1. Dissect tissue sample quickly and freeze in liquid nitrogen. Store at -70° to -80°C. Fresh tissue may also be used. Work very quickly and keep tissue on ice at all times including when tissue is weighed.
2. Add 5–10 mg (0.005–0.01 g) frozen ground tissue or fresh tissue to a 1.5 ml centrifuge tube containing 300 µl cell lysis solution, remove from ice, and homogenize thoroughly using a microfuge tube pestle. Place sample back on ice until next step.
3. Incubate lysate at 65°C for 15–60 min. Alternatively, if maximum yield is required, 1.5 µl proteinase K solution (20 mg/ml) may be added to the lysate. Mix by inverting capped tube 25 times and incubate at 55°C for 3 hr to overnight, until tissue particulates have dissolved. If possible, invert tube periodically during the incubation.

RNase Treatment

1. Add 1.5 µl RNase A solution (4 mg/ml) to the cell lysate.
2. Mix the sample by inverting the capped tube 25 times and incubate at 37°C for 15–60 min.

Protein Precipitation

1. Cool sample to room temperature.
2. Add 100 µl protein precipitation solution to the RNase A-treated cell lysate.
3. Vortex vigorously at high speed for 20 sec to mix the protein precipitation solution uniformly with the cell lysate. For samples with high polysaccharide content, incubate on ice for 5–15 min.
4. Centrifuge at 13,000–16,000 x g for 3 min. For samples with high polysaccharide content, centrifugation at 4°C may be required. The precipitated proteins should form a tight pellet. If the protein pellet is not tight, repeat step 3 and include the incubation on ice, then repeat step 4.

DNA Precipitation

1. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a 1.5 ml microfuge tube containing 300 µl 100% isopropanol (2-propanol).
2. Cap the tube and mix the sample by inverting gently 50 times.
3. Centrifuge at 13,000–16,000 x g for 1 min; the DNA will be visible as a small white pellet.
4. Pour off supernatant and drain tube briefly on clean absorbent paper. Add 300 µl 70% ethanol and invert capped tube several times to wash the DNA pellet.
5. Centrifuge at 13,000–16,000 x g for 1 min. Carefully pour off the ethanol. Pellet may be loose, so pour slowly and watch pellet to ensure it stays in the tube.
6. Invert and drain the tube on clean absorbent paper and allow to air-dry for 15 min.

DNA Hydration

1. Add 50 µl DNA hydration solution (50 µl will give a concentration of 100 µg/ml if the total yield is 5 µg DNA).
2. Allow DNA to rehydrate overnight at room temperature. Alternatively, heat at 65°C for 1 hr. Tap tube periodically to aid in dispersing the DNA. Store DNA at 2–8°C.

Ordering Information

Catalog #	Description
732-6340	AquaPure Genomic DNA Isolation Kit, for cultured cells and gram-negative bacteria, processes up to 100 cultured cell preps (1–2 x 10^6 cells/prep), or 100 x 0.5 ml bacterial cultures per kit
Related Products	
732-6343	AquaPure Genomic DNA Tissue Kit, for animal and plant tissues, cultured cells, and gram-negative bacteria, processes up to 100 x 0.5–10 mg animal and plant tissue preps, 100 cultured cell preps (1–2 x 10^6 cells/prep), or 100 x 0.5 ml bacterial cultures per kit
732-6345	AquaPure Genomic DNA Blood Kit, for human and mammalian whole blood and bone marrow, processes up to 100 x 0.3 ml whole blood samples per kit
732-6370	AquaPure RNA Isolation Kit, for cultured cells, animal and plant tissues, and gram-negative bacteria, processes up to 100 x 5–10 mg animal and plant tissue preps, 100 cultured cell preps (1–2 x 10^6 cells/prep), or 100 x 0.5 ml bacterial cultures per kit
732-6371	AquaPure RNA Blood Kit, for human and mammalian whole blood and bone marrow, processes up to 100 x 0.3 ml whole blood samples per kit

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