

Incubate at 65°C for 10 min (inverting several times to yield a homogeneous solution).

5. Add 220 µl **ethanol (96–100%)** to the lysate, and mix thoroughly by brief vortexing or inverting.
6. Pipet the mixture from step 5 into the spin column placed in a 2 ml collection tube (provided). Centrifuge at 12,000 rpm for 1 min. Discard flow-through.
 - Genomic DNA is adsorbed on the silica membrane of the column in this step.
7. Add 500 µl **Wash Buffer PS**, and centrifuge for 1 min at 12,000 rpm. Discard flow-through.
 - Wash Buffer PS must be diluted with isopropyl alcohol previously.
8. Add 500 µl **Wash Buffer PE**, and centrifuge for 1 min at 12,000 rpm. Discard flow-through.
 - Wash Buffer PE must be diluted with ethanol (96-100%) previously.
9. Repeat step 8.
10. Centrifuge for 3 min at 12,000 rpm to dry the column membrane. Discard flow-through and collection tube.
 - Since residual ethanol may interfere with subsequent reactions, it is important to dry the membrane of the spin column. This centrifugation step ensures that no residual ethanol will be carried during the following elution step. If carryover of ethanol occurs, empty the collection tube, then reuse it after centrifuging for 1 min at 12,000 rpm.
11. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl **Elution Buffer TE** directly onto the membrane. Incubate at room temperature for 2 min.
 - Elution buffer TE can be replaced by deionized water. But the pH should be 8.0-8.5.
 - Prewarm Elution Buffer TE to 65°C can increase the yield of genomic DNA.
12. Centrifuge for 2 min at 12,000 rpm. The tube contains the purified DNA. **Store the DNA at -20°C.**

Marine Animal Tissue Genomic DNA Extraction Kit

N1171 50 preps

N1172 100 preps

N1173 200 preps

For Research Use Only

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Kit Contents

Component	N1171	N1172	N1173
	50 preps	100 preps	200 preps
Solution DS	15 ml	30 ml	60 ml
Solution MS	20 ml	40 ml	80 ml
Proteinase K (20mg/ml)	1 ml	2 ml	4 ml
Wash Buffer PS	18 ml	36 ml	72 ml
Wash Buffer PE	15 ml	30 ml	30 ml × 2
Elution Buffer TE (10mM Tris-HCl, 1mM EDTA, pH 8.5)	5 ml	10 ml	20 ml
Spin Columns	50 each	100 each	200 each

Description

The genomic DNA extraction system uses the silica-based membrane technology for simple and fast isolation of genomic DNA (gDNA) without phenol/chloroform. Homogenization is not necessary since tissues are directly lysed by Proteinase K. The buffer system is optimized to allow selective binding of DNA to the silica-based membrane. The simple centrifugation procedure can completely remove impurities such as proteins, divalent cations, and secondary metabolites. Pure DNA is then eluted in water or low-salt buffer, ready to use.

The kit is suitable for high quality genomic DNA purification of tissue from marine animal, such as fish, shrimp, shellfish and crab, etc.

Downstream Applications

Purified DNA is free of impurities and enzyme inhibitors, and have an $A_{260/280}=1.7-1.9$, is suitable for applications such as:

- Genotyping
- PCR/qPCR
- Restriction enzyme digestion
- Sequencing
- Southern blotting

Features

- **Fast** - procedure takes only 30 min (not contain tissue processing)
- **High efficiency** - 3-35 μg of genomic DNA from 10 mg tissue
- **Safe** - no phenol/chloroform extraction step
- **High purity** - purified DNA without enzyme inhibitors, RNA or proteins, ready for downstream applications

Storage

Store Proteinase K at -20°C , other reagents at room temperature for up to 1 year.

Any precipitate in the Solution DS and Solution MS can be dissolved by incubating at 37°C before use.

Important Notes

- Prior to the initial use of the kit, dilute the Wash Buffer PS, Wash Buffer PE with isopropyl alcohol or ethanol (96-100%):

Solution	N1171 (50 preps)	N1172 (100 preps)	N1173 (200 preps)
Wash Buffer PS	18 ml	36 ml	72 ml
Isopropyl Alcohol	12 ml	24 ml	48 ml
Total Volume	30 ml	60 ml	120 ml

Solution	N1171 (50 preps)	N1172 (100 preps)	N1173 (200 preps)
Wash Buffer PE	15 ml	30 ml	30 ml (one bottle)
Ethanol	45 ml	90 ml	90 ml
Total Volume	60 ml	120 ml	120 ml

Mix well, mark the labels on the bottle that isopropyl alcohol or ethanol is added.

- Ensure that no DNases are introduced into the sterile solutions of the kit.
- Make sure there is no precipitates in Solution DS and Solution MS. If any precipitate is visible, warming the solutions at 37°C for 3-5 min to dissolve the precipitate, and cooling to 25°C before use.
- Wear disposable gloves when handling the Solution MS as it contains guanidine hydrochloride.
- All purification steps should be carried out at room temperature.
- All centrifugations should be carried out by a table-top microcentrifuge at $>12,000\text{ g}$ (10,000-14,000 rpm, depending on the rotor type).

Protocol

1. Marine animal tissue (fresh or frozen) can be processed by freezing with liquid nitrogen and grinding into powder using mortar and pestle. Add $\approx 10\text{ mg}$ of tissue powder to a 1.5 ml microcentrifuge tube.
2. Add 200 μl **Solution DS**. Mix immediately and thoroughly by brief vortexing or inverting.
Optional • If RNA-free genomic DNA is required, add 4 μl RNase A (100 mg/ml) and incubate for 5 min at room temperature. RNase A can be purchased separately.
3. Add 20 μl **Proteinase K**, Mix thoroughly by brief vortexing or inverting. Incubate at 55°C for several hours until yield a homogeneous solution (inverting several times during incubating).
4. Add 220 μl **Solution MS**, Mix thoroughly by brief vortexing or inverting.