EasyPure® Marine Animal Genomic DNA Kit

Cat. No. EE151

Storage: RNase A at -20°C for two years; others at room temperature (15-25°C) for one year

Description

EasyPure® Marine Animal Genomic DNA Kit provides a simple and convenient way to isolate high quality genomic DNA from up to 30 mg marine animals. DNA is bound to silica-based column. The isolated DNA is suitable for PCR, restriction enzyme digestion and Southern blot.

- DNA yield up to 40 µg.
- Complete removal of contaminants and inhibitors.
- Column based purification, no organic extraction or ethanol precipitation.

Sample requirement

Suitable for fresh or frozen tissue, avoid repeated freezing and thawing.

Kit Contents

Component	EE151-01 (50 rxns)
	EE151-11 (50 rxns)
Lysis Buffer 8 (LB8)	12 ml
Binding Buffer 8 (BB8)	9 ml
Clean Buffer 8 (CB8)	12 ml
Wash Buffer 8 (WB8)	12 ml
Elution Buffer (EB)	25 ml
RNase A (10 mg/ml)	1 ml (EE151-01)
	0 (EE151-11)
Proteinase K (20 mg/ml)	1 ml
Genomic Spin Columns with Collection Tubes	50 each

Procedures

Before start, add respective volume of 100% ethanol to BB8, CB8 and WB8.

All centrifugation steps are carried out at room temperature. Prepare 55°C and 70°C water bath in advance, respectively.

Component	**BB8**	**CB8**	**WB8**
50 rxns	27 ml	48 ml	48 ml

1. Place ≤30 mg minced tissue into a 1.5 ml sterile microcentrifuge tube.
2. Add 200 µl of LB8 and 20 µl of RNase A to the tube. Vortex for 10 seconds and incubate at room temperature for 2 minutes.
3. Add 20 µl of Proteinase K to the tube. Mix thoroughly by vortexing (ensure the tissue is completely immersed in solution). Incubate at 55°C until lysis is complete (the incubation time varies with tissues. For examples, 30 minutes for shellfish tissue, and 45 to 120 minutes for fish and shrimp). Vortexing is needed for thorough mix.
4. Add 1.5× volume of BB8 (check to make sure that ethanol has been added), mix thoroughly, apply to a spin column.
5. Centrifuge at 12,000×g for 30 seconds, discard the flow-through.
6. Add 500 µl of CB8 (check to ensure ethanol has been added), centrifuge at 12,000×g for 30 seconds, discard the flow-through.
7. Repeat step 6 once.

8. Add 500 µl of WB8 (check to ensure ethanol has been added), centrifuge at 12,000×g for 30 seconds, discard the flow-through.
9. Repeat step 8 once.
10. Centrifuge at 12,000×g for 2 minutes to completely remove residual WB8.
11. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 50-200 µl of Elution Buffer (for higher yield preheated the Elution Buffer to 60-70°C) or distilled water (pH >7.0) to the center of column. Incubate at room temperature for 2 minutes. Centrifuge at 12,000×g for 1 minute to elute the genomic DNA .
12. To better yield DNA, repeat step 11 once.

Notes

- For better DNA quality, use fresh sample and avoid repeated freezing and thawing.
- To avoid incomplete lysis, do not use too many starting materials.
- Cut tissues into smaller pieces for better lysis.

FOR RESEARCH USE ONLY