TIANamp Marine Animals DNA Kit

For isolation of genomic DNA from marine animal tissues

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TIANamp Marine Animals DNA Kit

(Spin Column)

Cat. no. 4992713/4992715

Kit Contents

Contents	4992713 50 preps	4992715 200 preps
Buffer GA	15 ml	50 ml
Buffer GB	15 ml	50 ml
Buffer GD	13 ml	52 ml
Buffer PW	15 ml	50 ml
Buffer TE	15 ml	60 ml
Proteinase K	1 ml	4 × 1 ml
Spin Columns CB3	50	200
Collection Tubes 2 ml	50	200
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Optional Reagents

RNaseA (100 mg/ml) (TIANGEN)

Storage

TIANamp Marine Animal DNA Kit can be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C. If a precipitate has formed in Buffer under 2-8°C, please place the buffer at room temperature or warm at 37°C for 10 min to dissolve the precipitate.

Introduction

TIANamp Marine Animal DNA Kit is based on silica membrane technology and the special buffer system can be used for the gDNA extraction from various samples. The spin column made of new type silica membrane can bind DNA optimally on given salt and pH conditions. Simple centrifugation completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Purified DNA is eluted in low-salt buffer or water, and is ready for use in downstream applications.

The genomic DNA isolated with this kit is of high quality and serves as an excellent template for agarose gel analysis, restriction enzyme digestion, PCR and Southern blot.

Extraction Yield

Sample	Suggested Incubation Time	DNA yield
Shellfish	0.5 hr	12-20 μg
Shrimp	1 hr	8-14 μg
Fish	1 hr	15-40 μg

Important Notes Before starting

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA yield and fragment size.
2. If a precipitate has formed in Buffer GA or Buffer GB, warm buffer at 37°C until the precipitate has fully dissolved.
3. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15-25°C).

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Preparation of samples

   Cut up to 30 mg tissue into small pieces and place in a 1.5 ml microcentrifuge tube, add 200 µl Buffer GA, vortex for 15 sec.

   Note: The sample from branchia is recommended not to exceed 20mg.

   Optional: If RNA removal is needed, add 4 µl of RNase A (100 mg/ml), mix by vortex, and incubate for 5 min at room temperature (15-25°C).

2. Add 20 µl Proteinase K (20 mg/ml), mix thoroughly by vortex. Briefly centrifuge to remove drops from the inside of the lid.

   Incubate at 56°C until it is completely lysed. Briefly centrifuge to remove drops from the inside of the lid.

   Note: Lysis time varies depending on the type of tissue processed, and usually will take 0.5-2 hr. For shellfish tissue, lysis usually needs 0.5 hr; for fish and shrimp tissue, lysis usually needs 1 hr. Samples should be inverted 2-3 times every one hour, mix thoroughly by vortex for 15 sec each time.

3. Add 200 µl Buffer GB to the sample, mix thoroughly by vortex, and incubate at 70°C for 10 min to yield a homogeneous solution. Briefly centrifuge to remove drops from the inside of the lid.

   Note: White precipitates may form when Buffer GB is added. They will not interfere with the procedure and will dissolve during the heat incubation at 70°C. If precipitates do not dissolve during heat incubation, it indicates that the cell is not completely lysed and may result in low yield and impure of DNA.

4. Add 200 µl ethanol (96-100%) to the sample, and mix thoroughly by vortex for 15 sec. A white precipitate may form by addition of ethanol. Briefly centrifuge to remove drops from the inside of the lid.

5. Pipet the mixture from step 4 into the Spin Column CB3 **(place CB3 in a 2 ml collection tube)** and centrifuge at 12,000 rpm (~13,400×g) for 30 sec. Discard flow-through and place the spin column into the collection tube.

6. Add 500 µl Buffer GD **(ensure ethanol (96-100%) has been added)** to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400×g) for 30 sec, then discard the flow-through and place the spin column into the collection tube.
7. Add 600 µl Buffer PW **(ensure ethanol (96-100%) has been added)** to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400×g) for 30 sec. Discard the flow-through and place the spin column into the collection tube.
8. Repeat Step 7.
9. Centrifuge at 12,000 rpm (~13,400×g) for 2 min to dry the membrane completely.

   Note: The residual ethanol of Buffer PW inhibits subsequent enzymatic reactions (e.g. restriction enzyme digestion and PCR).
10. Place the Spin Column CB3 in a new clean 1.5 ml microcentrifuge tube, and pipet 50-200 µl Buffer TE directly to the center of the membrane. Incubate at room temperature (15-25°C) for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400×g).

    Note: If the volume of elution buffer is less than 50 µl, it may affect recovery efficiency. The pH value of elution buffer will have a great effect on eluting, we suggest using buffer TE or distilled water (pH 7.0-8.5) to elute gDNA. For long-term storage of DNA, eluting in Buffer TE and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.