Ver. 260401

ODP343

Marine DNA Kit-Max

For extraction of genomic DNA from ethanol-fixed samples.

Origin®

ISO 13485:2016 ISO 9001:2015

Marine DNA Kit-Max

(ODP343)

Kit Contents

Contents	50 Preps
Buffer GS	10mL
Buffer EM	10mL
Buffer AB	5mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Proteinase K (20mg/mL)	1mL
Spin column CB3 with Collection Tubes (2mL)	50 Nos

Storage

Marine DNA Kit-Max should be stored for 1 year at room temperature (15-25°C). For long- term storage, the kit should be stored at 2-8°C.

Notes:

- **Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary.**
- **Buffer EM should be stored at 4°C.**
- **Proteinase K should be stored at -20°C.**

Introduction

Marine DNA Kit-Max is based on silica membrane technology and special buffer system for many kinds of sample's genomic DNA extraction. 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. Extracted DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

The genomic DNA isolated with this kit serves as an excellent template for agarose gel analysis, restriction enzyme digestion, PCR and blotting.

Important Notes

1. Add appropriate amount of ethanol (96-100%) to Buffer GD and Buffer PW as indicated on the bottle before use.
   Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.
2. All centrifugation steps are carried out at 13,400 ×g (12,000 rpm) in conventional tabletop microcentrifuge at room temperature.
3. Increasing the time of absorption and elution could improve recovery efficiency.
4. The recovery efficiency is related to starting DNA quantity and elution volume. The less starting quantity or elution volume, the less recovery efficiency.
5. If a precipitate has formed in Buffer GD, warm buffer to 56℃ until the precipitate has fully dissolved.

Protocol

- **Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle.**
- **Add 100µL Buffer AB to the center of spin column CB3, incubate at room temperature for 5 minutes and then centrifuge at 12,000 rpm for 2 minutes, before using the spin column.**

1. Preparation of samples:
   Cut up to 30mg tissue into small pieces and place in a 1.5mL microcentrifuge tube, add 200µL Buffer GS, vortex for 15 seconds. Homogenize the samples using micro pestle (before adding Proteinase K).
   Optional: RNase A (Cat# ORT405) treatment of the sample. Add 4µL RNase A (10mg/mL), mix by vortex, and incubate for 5 minutes at room temperature (15-25°C).
2. Add 20µL Proteinase K, mix thoroughly by vortexing. Incubate at 56°C until the tissue is completely lysed.
   Note: Lysis time varies depending on the type of tissue processed, usually will takes 0.5-2 hours. For ethanol fixed tissue, lysis usually needs 20-30 minutes. Samples should be inverted 2-3 times every 10 minutes.
3. Add 200µL Buffer EM to the sample, mix thoroughly by vortexing, and incubate at 30°C for 5 minutes to yield a homogeneous solution. Briefly, centrifuge the 1.5mL microcentrifuge tube at 1,000-3,000 rpm for 30 seconds to remove drops from the inside of the lid.
   Note: Precipitates are expected, but it will not interfere with the extraction.
4. Add 200µL chloroform to the sample. Cap the tube securely and mix the sample by inverting the tube for 15 seconds. Incubate for 3 minutes at room temperature. Centrifuge at 12,000 rpm (~13,400 ×g) for 10 minutes.
5. The mixture separates into a lower organic phase, and a colorless upper aqueous phase. DNA remains exclusively in the aqueous phase. Pipette out the aqueous phase into a new 1.5mL microcentrifuge tube.
   Note: Ensure not to disturb the organic phase.

6. Add 200µL ethanol (96-100%) to the sample, and mix thoroughly by inverting the tube for 20 seconds. Briefly centrifuge the 1.5mL microcentrifuge tube at 1,000 - 3,000 rpm for 30 seconds to remove drops from the inside of the lid. **Note: If any precipitates form, gently mix the solution to dissolve them. Before transferring the lysate to spin column CB3, add 100µL Buffer AB to the center of spin column CB3 (in a 2mL collection tube), incubate at room temperature for 5 minutes. Centrifuge at 12,000 rpm for 2 minutes and discard flow-through.**
7. Transfer the mixture from step 6 into the spin column CB3 (in a 2mL collection tube) and incubate the spin column for 5-10 minutes at room temperature. Centrifuge at 12,000 rpm (~13,400 ×g) for 45 seconds and discard flow-through. Place the spin column into the collection tube.
8. Add 500µL Buffer GD to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds, then discard the flow-through and place the spin column into the collection tube.
   Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.
9. Add 700µL Buffer PW to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes to remove the residual wash Buffer PW. Open the lid of the spin column CB3 and incubate at room temperature (15-25°C) for 2 minutes to dry membrane completely.
    Note: The resident ethanol of Buffer PW may have some affect in downstream application.
12. Place the spin column CB3 in a new clean 1.5mL microcentrifuge tube, and pipette 50-200µL Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15–25°C) for 2-5 minutes, and then centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g).
    Note: To increase the DNA yield, introduce the eluted Buffer TE to the column and centrifuge for 2 minutes at 12,000 rpm. **If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in elution, we suggest Buffer TE or distilled water (pH 7.0-8.5) to elute genomic DNA. 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.**