Ver: 1912

Marine Animals gDNA Isolation Kit

（BW-GD3311）

Contents

Kit Contents

Catalog#	BW-GD3311-00	BW-GD3311-01	BW-GD3311-02
Preps	4	50	250
DNA Mini Columns	4	50	250
2 mL Collection Tubes	4	50	250
Buffer HTL	1 mL	11 mL	55 mL
Buffer HBL	1 mL	12 mL	60 mL
Buffer KB	2.1 mL	26 mL	130 mL
DNA Wash Buffer*	2 mL	15 mL	3 x 24 mL
Elution Buffer	1 mL	11 mL	52 mL
Proteinase K	110 µL	1.3 mL	5×1.3 mL
User Manual	1	1	1

*Add 8 mL (BW-GD3311-00) or 60 mL (BW-GD3311-01) or 96 mL (BW-GD3311-02) absolute ethanol (96-100%) to each DNA Wash Buffer bottle before use.

Introduction

The key to this system is the new ezBind matrix that specifically, but reversibly, binds DNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or a low salt buffer.

The Marine Animails gDNA Isolation Kit provides an easy and rapid method for the isolation of genomic DNA for consistent PCR and southern analysis. Up to 30 mg of marine animal tissue, such as fish, shrimp, Scallop, Sea cucumber, can be readily processed at a time. This kit allows for the single or multiple simultaneous processing of samples. There is no need for phenol/chloroform extractions, and time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA can be directly used for most applications such as PCR, Southern Blotting, and Restriction Enzyme Digestion.

Storage and Stability

All Marine Animals gDNA Isolation Kit components are guaranteed for at least 12 months from the date of production when stored as follows:

Proteinase K is stable at room temperature (15-25℃) for one year. For long term, store aliquots at －20℃. Store all other contents at room temperature (15-25℃).

Before Starting

This kit is designed to be simple, fast, and reliable provided that all steps are followed diligently. It is strongly advised that you familiarize yourself with the entire user manual before starting.

Important Notes

✪ Add 8 mL (BW-GD3311-00) or 60 mL (BW-GD3311-01) or 96 mL (BW-GD3311-02) absolute ethanol (96-100%) to each DNA Wash Buffer bottle before use.

✪ Preheat Elution Buffer in 65℃.

✪*Carry out all centrifugations at room temperature.*

Materials not Supplied

✪ Tabletop microcentrifuge.

✪ Sterile 1.5 mL centrifuge tubes.

✪ Shaking water-bath set to 50℃.

✪ RNase A (20 mg/mL) (Optional).

✪ Absolute ethanol (96-100%).

Safety Information

Buffer HBL contains chaotropic salts. Use gloves and protective eyewear when handling this solution.

Marine Animals Genomic DNA Isolation Protocol (For spin)

This method is suitable for the isolation of genomic DNA from up to 30 mg of tissue. Yields vary depending on source.

Optional: Although mechanical homogenization of tissue is not required, pulverizing the samples in liquid nitrogen will improve lysis and reduce incubation time.

1. Mince up to 20-30 mg tissue and place it into a 1.5 mL microtube. Add **200 µL Buffer HTL.** In order to speed up lysis, cut the tissue into small pieces.

2. Add **25 µL Proteinase K.** Vortex to mix, and incubate in a shaking water bath set to 50℃ to effectively complete lysis. If no shaking water-bath is available, vortex the sample every 20-30 minutes**.**

   Note: Lysis time depends on the type of tissues. The average time is usually 1- 3 hours.

3. **Optional:** Certain tissues have high levels of RNA which will be co-purified with DNA using this kit. Add 4 µL RNase A and incubate at room temperature for 2 minutes.

4. Centrifuge for 5 minutes at 12,000 ×g to pellet insoluble tissue debris. Carefully aspirate the supernatant and transfer to a sterile microtube.

5. Add **220 µL Buffer HBL.** Vortex to mix. Incubate at 70℃ for 10 minutes.

   Note: A wispy precipitate may form upon addition of Buffer HBL, but does not interfere with DNA recovery. Adjust the volume of Buffer HBL required based on the amount of starting material.

6. Add 220 µL absolute ethanol (96-100%, room temperature). Vortex to mix.

   Note: Adjust the volume of ethanol required based on the amount of starting material.

7. Insert a **DNA Mini Column** into a **2 mL Collection Tube**. Transfer the entire sample from step **6** into the column including any precipitate that may have formed. Centrifuge at 10,000 ×g for 1 minute. Discard the flow-through.

8. Place the **DNA Mini Column** into the collection tube and add **500 µL Buffer KB.** Centrifuge at 10,000 ×g for 1 minute. Discard the flow-through.

9. Place the **DNA Mini Column** into the 2 **mL Collection Tube**. Add **600 µL DNA Wash Buffer** and centrifuge at 12,000 ×g for 1 minute. Discard the flow-through and reuse the collection tube.

10. Repeat step **9**.

11. Centrifuge the column, with the lid open**,** at maximum speed (12,000 ×g) for 2 minutes to remove the residual ethanol.

    Note: This step is critical for optimal DNA elution.

12. Place the **DNA Mini Column** into a sterile 1.5 mL microtube, and add **100-200 µL** preheated (65℃) **Elution Buffer**. Allow to sit at room temperature for 1-3 minutes. Centrifuge at 12,000 ×g for 1 minute to elute DNA.

13. **Optional:** Repeat the elution with a second **100-200 µL Elution Buffer**.

    Note: Each 200 µL elution typically produces yields of 60-70% of the DNA. Thus two elution will generally give 90% yields. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL of Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances, yields may be increased by incubating the column at 65℃ (rather than at room temperature) upon the addition of Elution Buffer.

Protocol For spin/vacuum

Carry out disruption, homogenization, proteinase digestion, and loading onto the Mini Column as indicated in previous protocols. Instead of continuing with centrifugation, follow the steps outlined below.

Note: Please read through previous sections of this manual before beginning this protocol.

1. Prepare the vacuum manifold according to manufacturer's instruction, and connect the **DNA Mini Column** to the manifold.

2. Load the sample onto the column.

3. Switch on vacuum to draw the sample through the column, and then turn off the vacuum.

4. Wash the column by adding **500 µL Buffer KB.** Draw the liquid through the column by turning on the vacuum.

5. Wash the column by adding **600 µL DNA Wash Buffer**. Draw the Wash Buffer through the column by turning on the vacuum source.

6. Washing the column with another **600 µL DNA Wash Buffer.**

7. Place the column back to the **2 mL Collection Tube**. Spin for 2 minutes at maximum speed with the lid open to dry the column.

8. Place the column into a clean 1.5 mL microtube, and add **100-200 µL** of preheated (65°C) **Elution Buffer.** Let it sit at room temperature for 1-2 minutes, and then centrifuge at 12,000 ×g for 1 minute to elute DNA.

   Optional: Repeat the elution with a second **100-200 µL Elution Buffer**.

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl, or Elution Buffer as a blank. DNA concentration is calculated as:

[DNA] = (Absorbance260) x (0.05 µg/µL) x (Dilution Factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and 280 nm. An A_{260}/A_{280} ratio of 1.7-1.9 corresponds to 85%-95% purity.

Expected yields vary with both amount and type of tissue used.

Typically, 30 mg of fresh tissue will yield 10~40 µg of DNA with two elutions (each 200 µL).

water or 10 mM Tris-HCl, pH 8.5.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Clogged column	Incomplete lysis.	Extend incubation time of lysis with Buffer HTL, and Proteinase K. Add the correct volume of Buffer HBL and incubate for specified time at 70℃. It may be necessary to extend incubation time by 10 minutes.
	Sample is too large.	If using more than 30 mg tissue, increase volumes of Proteinase K, Buffer HTL, Buffer HBL, and ethanol. Pass aliquots of lysate through one column successively.
	Sample is too viscous.	Divide sample into multiple tubes, and adjust the volume to 25 µL with Buffer HTL.
	Sample has low DNA content.	Increase starting material and volume of all reagents (Proteinase K, Buffer HTL, Buffer HBL, and ethanol) proportionally. Pass aliquots of lysate through column successively.
	Extended centrifugation during elution.	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation-it will not interfere with PCR or restriction digests.
Low A_{260}/A_{280} ratio	Poor cell lysis due to incomplete mixing with Buffer HBL.	Repeat the procedure, this time making sure to vortex the sample with Buffer HBL immediately and completely.
	Incomplete cell lysis or protein degradation due to insufficient incubation.	Increase incubation time with Buffer HTL, and Proteinase K. Ensure that no visible pieces of tissue remain.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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