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Mollusc gDNA Isolation Kit

（BW-GD2414）

Contents

Kit Contents

Catalog#	BW-GD2414-00	BW-GD2414-01	BW-GD2414-02
Preps	4	50	250
DNA Mini Columns	4	50	250
2 mL Collection tubes	4	50	250
Buffer MTL	2 mL	20 mL	90 mL
Buffer MBL	2 mL	20 mL	100 mL
Buffer KB	3 mL	26 mL	130 mL
Proteinase K	150 µL	1.3 mL	5 x 1.3 mL
RNase A (20 mg/mL)	15 µL	120 µL	550 µL
DNA Wash Buffer*	2 mL	15 mL	3 x 24 mL
Elution Buffer	2 mL	15 mL	30 mL
User Manual	1	1	1

* Add 8 mL (BW-GD2414-00) or 60 mL (BW-GD2414-01) or 96 mL (BW-GD2414-02) of absolute ethanol to each bottle.

Introduction

The Mollusc gDNA Isolation Kit is designed for efficient recovery of genomic DNA from Molluscs, arthropods, roundworms, and flatworms. The method is suitable for samples frozen or preserved in alcohol or DNE solution, and good results can be obtained with formalin preserved material.

Samples are homogenized and lysed in a high salt buffer and extracted with chloroform to remove polysaccharides. Following a rapid alcohol precipitation step, binding conditions are adjusted and DNA further purified using DNA spin columns. In this way, salts, proteins and other contaminants are removed to yield high quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization techniques.

Storage and Stability

All Mollusc 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

Please read the entire booklet to become familiar with the Mollusc gDNA Isolation Kit protocol.

Dilute DNA Wash Buffer concentrate with absolute ethanol as follows and store at room temperature.

- BW-GD2414-00: Add 8 mL (96%-100%) ethanol to each bottle.
- BW-GD2414-01: Add 60 mL (96%-100%) ethanol to each bottle.
- BW-GD2414-02: Add 96 mL (96%-100%) ethanol to each bottle.

Materials to be provided by user

- Microcentrifuge capable of at least 12,000 x g.
- Nuclease-free 1.5 mL or 2 mL microfuge tubes.
- Water bath equilibrated to 65-70 ℃.
- Equilibrate Elution Buffer at 65℃.
- Absolute (96%-100%) ethanol.
- chloroform:isoamyl alcohol (24:1).

Mollusc samples preserved in formalin should be rinsed in xylene and then ethanol before processing. Note that results obtained with formalin-fixed tissues generally depend on age and size of specimen. Purified material is usually adequate for PCR amplification, but fresh or frozen samples should be used for southern analysis.

Mollusc gDNA Isolation Protocol

Molluscs

1. Pulverize no more than 30 mg of tissue in liquid nitrogen with mortar and pestle and place the powder in a clean 1.5 mL microcentrifuge tube. If ceramic mortar and pestle are not available, homogenize the sample in the microfuge tube using a disposable microtube pestle. Proceed to step **2** below.

Arthropods and other soft tissue invertebrates

1. **Grind no more than 30 mg tissue in liquid nitrogen** with mortar and pestle and place the powder in a clean 1.5 mL microcentrifuge tube. If ceramic mortar and pestle are not available, homogenize the sample in the microfuge tube using a disposable microtube pestle. Addition of a pinch of white quartz sand, 50 to 70 mesh (Sigma Chemical Co. Cat No. S9887) will help. Proceed to step **2** below.
2. Add **350 µL Buffer MTL** followed by **25 µL Proteinase K** (20 mg/mL). Vortex briefly to mix and incubate at 65°C for a minimum of 30 minutes or until entire sample is solubilized. Actual incubation times vary and depend on elasticity of tissues. Most samples require no more than 4 hours. Alternatively an overnight incubation at 37°C will produce adequate results.
3. To the lysate, add 350 µL chloroform:isoamyl alcohol (24:1) and vortex for 5 seconds to mix. Centrifuge at 10,000 x g for 2 minutes at room temperature. Carefully transfer the upper aqueous phase to a clean 1.5 mL microfuge tube. Avoid the milky interface containing contaminants and inhibitors.

Note: This step removes polysaccharides and proteins from the solution and improves spin-column performance downstream. If very few upper aqueous phase present after centrifugation, add 200 µL of Buffer MTL and mix well by vortexing. Centrifuge as above and transfer the upper aqueous phase to a clean 1.5 mL tube.

4. Add **1 volume** of **Buffer MBL** followed by **2 µL RNase A** (20 mg/mL), vortex at maxi speed for 15 seconds. Incubate at 70°C for 10 minutes.
5. Add 1 volume of absolute ethanol (room temperature, 96-100%) and mix well by vortexing at maxi speed for 15 seconds.

Tips: **500 µL** upper aqueous solution, add **500 µL Buffer MBL** and 500 µL of absolute ethanol.

6. Apply **750 µL** of the mixture from step **5**, including any precipitation that may have formed, to the DNA column. Centrifuge at 10,000x g for 1 minute at room temperature. Discard

flow-through liquid and re-use collection tube.

7. Apply the remaining of mixture into the column and centrifuge as above. Discard flow-through liquid and put the column back to the collection tube.
8. Add **500 µL Buffer KB** to the column. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through liquid and re-use collecting tube in next step.
9. Place column into the collection tube and wash by adding **600 µL DNA Wash Buffer** (add absolute ethanol before use). Centrifuge at 10,000 x g for 30 seconds. Discard flow-through liquid and re-use collecting tube in next step.

Note: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle and page 4. If refrigerated, the diluted DNA wash buffer must be brought to room temperature before use.

10. Repeat step **9** with another **600 µL DNA Wash Buffer** (add absolute ethanol before use)..Discard liquid and re-insert the column, **with the lid open**, to the empty collecting tube.
11. Centrifuge the column at 12,000 x g for 2 minutes to remove the residual ethanol.
12. Place column into a clean 1.5 mL microfuge tube (not suplied). To elute DNA add **50 -100 µL** of **Elution Buffer** or ddH_2O preheated to 65°C directly onto the matrix. Allow soaking for 2 minutes at room temperature. Centrifuge at 10,000 x g for 1 minute to Elute DNA.

Optional: Add the eluted DNA back to the column for a second elution normally yields another 20-30% of the DNA while the first elution typically yields 60-70% of the DNA.

Elution volume < 50 µL greatly reduces yields. Yields may be increased by incubating the column at 65°C (rather than at room temperature) upon the addition of Elution Buffer.

Determination of DNA Quality and Quantity

Dilute a portion of the eluted material approximately 10-20 fold in DNA Elution Buffer or 10 mM Tris, pH 8.0. Measure absorbance at 280 nm and at 260 nm to determine the A260/A280 ratio. Values of 1.7-1.9 generally indicate 85%-90% purity. The concentration of DNA eluted can be determined as follows:

Concentration = 50 ug/mL x Absorbance260 x {Dilution Factor}

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
No DNA eluted	Poor cell lysis.	Increase incubation time with Buffer MTL. An overnight incubation may be necessary.
	Incomplete homogenization	Pulverize starting material as indicated in liquid nitrogen to obtain a fine powder.
	Absolute ethanol not added before adding sample to column.	Before applying DNA sample to column, add Buffer MBL and absolute ethanol.
	No ethanol added to DNA Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before first use

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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