E.Z.N.A.® Mollusc DNA Kit

D3373-00	5 preps
D3373-01	50 preps
D3373-02	200 preps

May 2013

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Manual Revision : May 2013

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The E.Z.N.A.® Mollusc DNA Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from molluscs, insects, arthropods, roundworms, flatworms, and other invertebrate tissue samples rich in mucopolysaccharides. The method is suitable for invertebrates frozen or preserved in alcohol or DNE solution, and good results can be obtained with formalin preserved material.

The procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding of Omega Bio-tek's HiBind® matrix. Samples are homogenized and lysed in a high salt buffer containing CTAB and extracted with chloroform to remove mucopolysaccharides. Binding conditions are adjusted and DNA further purified using HiBind® 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.

New in this Edition:

- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D3373-00	D3373-01	D3373-02
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
ML1 Buffer	3 mL	30 mL	125 mL
MBL Buffer	5 mL	20 mL	80 mL
HBC Buffer	3 mL	25 mL	80 mL
Proteinase K Solution	150 µL	1.4 mL	4 x 1.4 mL
RNase A	55 µL	550 µL	2 x 1.1 mL
DNA Wash Buffer	1.5 mL	15 mL	3 x 20 mL
Elution Buffer	1 mL	20 mL	50 mL
User Manual	1	1	1

Storage and Stability

All of the E.Z.N.A.® Mollusc DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. RNase A should be stored at 2-8°C. Proteinase K Solution can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K Solution at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

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 8.5. 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 µg/mL x Absorbance260 x {Dilution Factor}

Preparing Reagents

1. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3373-00	6 mL
D3373-01	60 mL
D3373-02	80 mL per bottle

2. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D3373-00	1.2 mL
D3373-01	10 mL
D3373-02	32 mL

E.Z.N.A.® Mollusc DNA Protocol

Invertebrates 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 analyses.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 10,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water baths capable of 60°C and 70°C
- Vortexer
- 100% ethanol
- Chloroform:isoamyl alcohol (24:1)

Before Starting:

- Prepare buffers according to the instructions on Page 4
- Set water baths to 60°C and 70°C
- Heat Elution Buffer to 70°C

1. Homogenize tissue sample following one of the procedures below depending on the sample type.

   A. Arthropods
      1. Pulverize no more than 50 mg of tissue in liquid nitrogen with a mortar and pestle.

      Note: If ceramic mortar and pestle are not available, homogenize the sample in the microcentrifuge tube using a disposable microtube pestle (Omega Bio-tek, Cat No. SSI-1015-39; Eppendorf, Cat No. 0030 120.973; VWR, Cat No. KT 749520-0000).

      2. Transfer the powder to a clean 1.5 mL microcentrifuge tube.

      3. Proceed to Step 2 below.

B. Molluscs (and other soft tissue invertebrates)

1. Pulverize no more than 30 mg tissue in liquid nitrogen with a mortar and pestle.

2. Transfer the powder to a clean 1.5 mL microcentrifuge tube.

   Note: If ceramic mortar and pestle are not available, homogenize the sample in the microcentrifuge tube using a disposable microtube pestle (Omega Bio-tek, Cat No. SSI-1015-39; Eppendorf, Cat No. 0030 120.973; VWR, Cat No. KT 749520-0000). Addition of a pinch of white quartz sand, -50 to 70 mesh (Sigma Chemical Co. Cat No. S9887) will help.

3. Proceed with Step 2 below.

Note: The amount of starting material depends on the sample and can be increased if acceptable results are obtained with the suggested 30 mg tissue. For easy-to-process specimens, the procedure may be scaled up and the buffer volumes used increased in proportion. In any event, use no more than 50 mg tissue per HiBind® DNA Mini Column as binding capacity (100 µg) may be exceeded. Meanwhile, difficult tissues may require starting with less than 30 mg tissue and doubling all volumes to ensure adequate lysis.

2. Add 350 µL ML1 Buffer and 25 µL Proteinase K Solution. Vortex to mix thoroughly.

3. Incubate at 60°C for a minimum of 30 minutes or until entire sample is solubilized.

   Note: Actual incubation time varies and depends on the elasticity of the tissue. Most samples require no more than 4 hours. Alternatively an overnight incubation at 37°C will produce adequate results.

4. Add 350 µL chloroform:isoamyl alcohol (24:1). Vortex to mix thoroughly.

5. Centrifuge 10,000 x *g* for 2 minutes at room temperature.

6. Transfer the upper aqueous phase to a clean 1.5 mL microcentrifuge tube. Avoid the milky interface containing contaminants and inhibitors.

   Note: This step will remove much of the polysaccharides and proteins from solution and improve spin-column performance downstream. If a small upper aqueous phase is present after centrifugation, add 200 µL ML1 Buffer and vortex to mix thoroughly. Repeat Step 5 (centrifugation) and Step 6 (transfer the upper aqueous phase).

7. Add one volume MBL Buffer and 10 µL RNase A. Vortex at maximum speed for 15 seconds.

   Note: For example, to 500 µL upper aqueous solution from Step 6, add 500 µL MBL Buffer.

8. Incubate at 70°C for 10 minutes.

9. Cool the sample to room temperature.

10. Add one volume 100% ethanol. Vortex at maximum speed for 15 seconds.

    Note: For example, to 500 µL upper aqueous solution from Step 6, add 500 µL 100% ethanol.

11. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Centrifuge at maximum speed for 60 seconds.
3. Discard the filtrate and reuse the collection tube.

12. Transfer 750 µL sample from Step 9 (including any precipitate that may have formed) to the HiBind® DNA Mini Column.

13. Centrifuge at 10,000 x *g* for 1 minute.

14. Discard the filtrate and reuse the collection tube.

15. Repeat Steps 12-14 until all of the sample has been applied to the HiBind® DNA Mini Column.

16. Discard the filtrate and the Collection Tube.

17. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

18. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

19. Centrifuge at 10,000 x *g* for 30 seconds.

20. Discard the filtrate and reuse the Collection Tube.

21. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please refer to Page 4 or the bottle label for instructions.

22. Centrifuge at 10,000 x *g* for 1 minute.

23. Discard the filtrate and reuse the Collection Tube.

24. Repeat Steps 21-23 for a second DNA Wash Buffer wash step.

25. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the membrane.

    Note: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.

26. Transfer the HiBind® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).

27. Add 50-100 µL Elution Buffer (or sterile deionized water) preheated to 70°C.

    Note: Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

28. Let sit at room temperature for 2 minutes.

29. Centrifuge at 10,000 x *g* for 1 minute.

30. Repeat Steps 27-29 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

31. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff , toll free, **at (800-832-8896).**

Problem	Cause	Solution
Clogged Column	Incomplete lysis	Increase incubation time with ML1 Buffer/ Proteinase K Solution. An overnight incubation may be necessary.
	Sample too large	Do not use greater than the recommended amount of starting material. For larger samples, divide into multiple tubes.
	Incomplete homogenization	Pulverize material as indicated in liquid nitrogen to obtain a fine powder.
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume. Incubate the column at 70°C for 5 minutes before centrifugation.
	Poor binding to column	Follow the protocol closely when adjusting the binding conditions.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol before use.
Low DNA Yield or no DNA Yield	Extended centrifugation during elution step	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.
	DNA washed off	Repeat the DNA isolation, be sure to thoroughly mix the sample with ML1 Buffer.
	Trace protein contaminants remain	Following Step 19, wash column with a mixture of 300 µL MBL Buffer and 300 µL ethanol before proceeding to Step 20.
No DNA eluted	Poor cell lysis	Increase incubation time with ML1 Buffer. An overnight incubation may be necessary.
	Incomplete homogenization	Pulverize starting material as indicated in liquid nitrogen to obtain a fine powder.
	100% ethanol was not added before adding sample to column	Before applying DNA sample to column, add MBL Buffer and 100% ethanol.
	Ethanol was not added to the DNA Wash Buffer	Dilute DNA Wash Buffer with the indicated volume of 100% ethanol before use.
	Isopropanol was not added to the HBC Buffer	Dilute HBC Buffer with the indicated volume of isopropanol before use.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
RNAse A, 5 mL	PD090
Proteinase K Solution	AC116
Elution Buffer, 100 mL	PDR048
DNA Wash Buffer, 100 mL	PS010

Notes: