omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Mollusc & Insect DNA Kit

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

Manual Date: January 2024
Revision Number: v1.0

For Research Use Only

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek

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Table of Contents

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Intended Use

For professional research use.

The E.Z.N.A.® Mollusc & Insect DNA Kit is intended for isolation and purification of genomic DNA from molluscs, insects, arthropods, roundworms, flatworms, and other invertebrate tissue samples rich in mucopolysaccharides.

Intended User

The E.Z.N.A.® Mollusc & Insect DNA Kit is intended for professional use and to be used by or under the supervision of professional users, such as laboratory personnel, technicians, researchers and physicians specifically instructed and trained in molecular biology techniques.

Product Description

The E.Z.N.A.® Mollusc & Insect DNA Kit is designed for efficient recovery of genomic DNA from molluscs, insects, arthropods, roundworms, flatworms, and other invertebrate tissue samples rich in mucopolysaccharides. This kit can also be used with formalin preserved material or invertebrates frozen or preserved in alcohol or DNE solution.

The procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding technology 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. Following a rapid alcohol precipitation step, DNA is bound to the HiBind® DNA Mini Column upon adjustment of binding conditions and further purified through multiple wash steps. 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:

January 2024

- In the newest version of this kit, the E.Z.N.A.® Mollusc DNA Kit (D3373) and E.Z.N.A.® Insect DNA kit (D0926) have merged.
- CTL Buffer has been discontinued and replaced with ML1 Buffer. This is a name change only; the formulation has not changed.

Kit Contents

Product	D3373-00	D3373-01	D3373-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
ML1 Buffer	3 mL	30 mL	125 mL
BL Buffer	5 mL	20 mL	80 mL
HBC Buffer	5 mL	25 mL	80 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL
Elution Buffer	2 mL	30 mL	60 mL
Proteinase K Solution	150 µL	1.5 mL	6.0 mL
RNase A	55 µL	520 µL	2.1 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Mollusc & Insect DNA Kit components are guaranteed for at least 12 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 and away from bright light. 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 A260 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	10 mL
D3373-01	100 mL
D3373-02	100 mL per bottle

2. Dilute HBC Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
D3373-00	2.0 mL
D3373-01	10 mL
D3373-02	32 mL

Warnings

This kit is for research use only.

Please read all instructions carefully before using the kit.

Decontaminate and dispose of all potentially infectious materials in accordance with applicable local, state, and national regulations. Please refer to safety data sheets (SDSs) for information on disposal of different components included in this kit.

Safety Information

All chemicals and biological materials are potentially hazardous. Biological samples such as plasma, serum, tissues, body fluids, blood etc. are potentially infectious and must be treated as biohazardous materials. Conduct all work in properly equipped facilities following universal precautions and using appropriate personal safety equipment such as disposable gloves, lab coats, safety glasses etc. as required by policies and procedures outlined by your facility. Please refer to safety data sheets (SDSs) for information on safe handling, transport and disposal of different components included in this kit. SDSs are made available in PDF format on the product page at www.omegabiotek.com. Discard all waste in accordance with the local safety regulations.

Some of the buffers included in the product contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions to guanidine-containing waste.** Please access the SDSs online for detailed information on the reagents.

Precautions

Some of the buffers included in the E.Z.N.A.® Mollusc & Insect DNA Kit contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions** to guanidine containing sample-preparation waste. Please access the SDSs online for detailed information on the reagents.

Component	Description
RNase A 	Contains: Ribonuclease A. Danger! Causes an allergic skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection in case of inadequate ventilation. Contaminated work clothing must not be allowed out of the workplace. INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a poison center/doctor/physician/first aider if experience respiratory symptoms. ON SKIN: Wash with plenty of water. Get medical attention if skin irritation or rash occurs. Take off contaminated clothing and wash it before reuse.
BL Buffer 	Contains: Guanidine hydrochloride. Warning! Causes serious eye irritation. Harmful if swallowed. Causes skin irritation. May cause respiratory irritation. Do not eat, drink or smoke when using this product. Wear protective gloves/protective clothing/eye protection/face protection. INHALED: Remove person to fresh air and keep comfortable for breathing. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Get medical advice/attention if eye irritation persists. ON SKIN: Wash with plenty of water and soap. Get medical advice/attention if skin irritation occurs. SWALLOWED: Rinse mouth. Call a poison center or doctor/physician if you feel unwell.
HBC Buffer 	Contains: Guanidine hydrochloride. Warning! Causes serious eye irritation. Harmful if swallowed. Causes skin irritation. Do not eat, drink or smoke when using this product. Wear protective gloves/protective clothing/eye protection/face protection. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention if eye irritation persists. Take off contaminated clothing and wash before resuse. ON SKIN: Wash with plenty of water and soap. Get medical advice/attention if skin irritation occurs. SWALLOWED: Rinse mouth. Call a poison center or doctor/physician if you feel unwell.

Precautions

Component	Description
ML1 Buffer 	Contains: Cetyltrimethyl ammonium bromide (CTAB). Warning! Causes serious eye irritation. Causes skin irritation. Do not eat, drink or smoke when using this product. Wear protective gloves/protective clothing/eye protection/face protection. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention if eye irritation persists. Take off contaminated clothing and wash before resuse. ON SKIN: Wash with plenty of water and soap. Get medical advice/attention if skin irritation occurs.
Proteinase K Solution 	Contains: Proteinase K. Danger! May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear respiratory protection in case of inadequate ventilation. INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a poison center/doctor/ physician/first aider if experience respiratory symptoms.

E.Z.N.A.® Mollusc & Insect 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*g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water baths capable of 60°C and 70°C
- Vortexer
- 100% ethanol
- 100% isopropanol
- Chloroform:isoamyl alcohol (24:1)
- Optional: 3M NaOH for column equilibration

Before Starting:

- Prepare buffers according to the instructions on Page 5
- Set water baths to 60°C and 70°C
- Heat Elution Buffer to 70°C

1. Prepare samples using one of the procedures below depending on the sample type.

   A. Insects and 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 (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.

      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.

   Note: If ceramic mortar and pestle are not available, homogenize the sample in the microcentrifuge tube using a disposable microtube pestle (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.

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

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 amount of 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*g* for 2 minutes at room temperature.

6. Carefuly 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 BL Buffer and 10 µL RNase A. Vortex at maximum speed for 15 seconds.

   Note: For example, to 350 µL upper aqueous solution from Step 6, add 350 µL BL 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 350 µL upper aqueous solution from Step 6, add 350 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 10 (including any precipitate that may have formed) to the HiBind® DNA Mini Column.

13. Centrifuge at 10,000*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 100% isopropanol before use. Please see Page 5 for instructions.

19. Centrifuge at 10,000*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 before use. Please see Page 5 for instructions.

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

23. Discard the filtrate and reuse the collection tube.

24. Repeat Steps 21-23 for a second DNA Wash Buffer 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 for 2 minutes at room temperature.

29. Centrifuge at 10,000*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 BL Buffer and 300 µL ethanol before proceeding to Step 20.

Troubleshooting Guide

Problem	Cause	Solution
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 BL 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.
Problems in downstream applications	RNA contamination	Double the RNase A volume (Step 7) and incubation time (Step 8) on Page 11.
		Remove RNA from eluted DNA sample by following the steps outlined below. More components may need to be purchased separately. • Add 10 µL RNase A to the eluted DNA and incubate for 5 minutes at room temperature. • Add one volume 100% ethanol and one volume BL Buffer. Vortex briefly to mix. • Insert a HiBind DNA Mini Column into a 2 mL Collection tube. • Transfer solution to HiBind DNA Mini Column and follow steps 13-31 to rebind, wash, and elute DNA from column.

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling:

Picture	Description
	No additional hazards or not classified as hazardous according to GHS
 MM/DD/YYYY	Use-by date
	Check components for storage conditions.
	Lot number
	Manufacturer
	Website
	Telephone
	Fax
	Email
	LinkedIn
	Twitter
	Facebook

For more purification solutions, visit **www.omegabiotek.com**

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