E.Z.N.A.® Insect DNA Kit

Table of Contents

Manual Revision: May 2013

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction and Overview

The E.Z.N.A.® Insect DNA Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from insects, arthropods, roundworms, flatworms, and some plant tissue samples rich in polysaccharides. The method is suitable for frozen samples or for samples preserved in alcohol or DNE solution. Good results also 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 ability of Omega Bio-tek's HiBind® matrix, to isolate high-quality DNA.

Samples are homogenized and lysed in a high salt buffer containing CTAB and extracted with chloroform to remove polysaccharides. Following a rapid alcohol precipitation step, binding conditions are adjusted and DNA is further purified using HiBind® DNA Mini 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:

- This manual has been edited for content and redesigned to enhance user readability.
- HB Buffer has been replaced by HBC Buffer.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	D0926-00	D0926-01	D0926-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
CTL Buffer	2 mL	20 mL	80 mL
CBL Buffer	3 mL	30 mL	120 mL
HBC Buffer	3 mL	25 mL	80 mL
Proteinase K Solution	150 µL	1.4 mL	4 x 1.4 mL
RNase A	12 µL	120 µL	450 µL
DNA Wash Buffer	1.5 mL	15 mL	3 x 20 mL
Elution Buffer	1 mL	20 mL	50 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Insect DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. RNase A must 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 of the buffers. Dissolve such deposits by warming the solution at 65°C and gently shaking.

Preparing Reagents

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

Kit	100% Ethanol to be Added
D0926-00	6 mL
D0926-01	60 mL
D0926-02	80 mL per bottle

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

Kit	Isopropanol to be Added
D0926-00	1.2 mL
D0926-01	10 mL
D0926-02	32 mL

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

DNA concentration = Absorbance 260 × 50 × (Dilution Factor) µg/mL

A ratio greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) sometimes can be determined best by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatemers may also be present.

E.Z.N.A.® Insect DNA Kit Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water baths capable of 70°C
- 100% ethanol
- Isopropanol
- Chloroform
- Isoamyl alcohol
- Optional: 3M NaOH
- Optional: Sterile deionized water or 10 mM Tris, pH 9.0

Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the instructions in the Preparing Reagents section on Page 4
- Prepare a 24:1 solution of chloroform:isoamyl alcohol
- Heat water baths to 60°C and 70°C
- Heat Elution Buffer to 70°C

Insect samples preserved in formalin should be rinsed in xylene and ethanol before processing. 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.

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

   A. Insects

      i. Pulverize no more than 50 mg tissue in liquid nitrogen using a mortar and pestle.

         Note: If a 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 & SSI-1014-39).

      ii. Transfer the powdered tissue to a clean 1.5 mL microcentrifuge tube (not provided).

      iii. Proceed to Step 2 below.

B. Arthropods (and other soft tissue invertebrates and plant samples)

i. Pulverize no more than 30 mg tissue in liquid nitrogen using a mortar and pestle.

Note: If a ceramic mortar and pestle are not available, homogenize the sample in the microcentrifuge tube using a disposable microtube pestle (Omega Bio-tek, Cat# SSI-1015-39 & SSI-1014-39). Addition of a pinch of white quartz sand, -50 to 70 mesh (Sigma Chemical Co., Cat No. S9887) will help.

ii. Transfer the powdered tissue to a clean 1.5 mL microcentrifuge tube (not provided).

iii. Proceed to Step 2 below.

Note: 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 volumes of all buffers used increased in proportion. In any event, use no more than 50 mg tissue per HiBind® DNA Mini Column as the DNA binding capacity (100 µg) may be exceeded. Difficult tissues may require starting with less than 30 mg tissue and doubling all volumes to ensure adequate lysis.

2. Add 350 µL CTL Buffer and 25 µL Proteinase K Solution. Vortex briefly to mix.

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

Note: Actual incubation times may vary and depend on the elasticity of tissues. 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 at 10,000 x *g* for 2 minutes at room temperature.

6. Carefully transfer the upper aqueous phase to a clean 1.5 mL microcentrifuge tube (not provided). 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 very little upper aqueous phase is present after centrifugation, add 200 µL CTL1 Buffer. Vortex to mix thoroughly. Repeat Steps 5-6 above.

7. Add one volume CBL Buffer and 2 µL RNase A. Vortex at maximum speed for 15 seconds.

8. Incubate at 70°C for 10 minutes.

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

   Note: For example, for 500 µL upper aqueous solution, add 500 µL CBL Buffer and 500 µL 100% ethanol.

10. 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. Let sit for 4 minutes.
3. Centrifuge at maximum speed for 20 seconds.
4. Discard the filtrate and reuse the Collection Tube.

11. Transfer 750 µL cleared lysate, including any precipitates that may have formed, from Step 9 by CAREFULLY aspirating it into the HiBind® DNA Mini Column.

12. Centrifuge at maximum speed for 1 minute.

13. Discard the filtrate and reuse the collection tube.

14. Repeat Steps 11-13 until all the remaining samples has been transferred to the HiBind® DNA Mini Column.

15. Transfer the HiBind® DNA Mini Column into a 2 mL Collection Tube.

16. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

17. Centrifuge at maximum speed for 30 seconds.

18. Discard the filtrate and reuse collection tube.

19. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

20. Centrifuge at maximum speed for 1 minute.

21. Discard the filtrate and reuse the collection tube.

22. Repeat Steps 19-21 for a second DNA Wash Buffer wash step.

23. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

24. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

25. Add 50-100 µL Elution Buffer, sterile deionized water, or 10 mM Tris, pH 9.0 heated to 70°C directly to the center of the column membrane.

26. Let sit at room temperature for 2 minutes.

27. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

28. Repeat Steps 25-27 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).

29. 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 **1-800-832-8896**.

Problem	Cause	Solution
Clogged Column	Incomplete lysis	Increase incubation time with CTL Buffer and Proteinase K Solution. An overnight incubation may be necessary.
	Sample too large	Do not use more than the recommended amount of starting material. For larger samples, divide into multiple tubes.
	Incomplete homogenization	Pulverize starting material in liquid nitrogen as indicated to obtain a fine powder.
Problem	Cause	Solution
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume.
	Poor binding to column	Follow protocol closely when adjusting the binding conditions.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol. See Page 4 for instructions.
		HBC Buffer must be diluted with isopropanol. See Page 4 for instructions.
	100% ethanol not added before adding sample to column	Before applying DNA sample to column, add CBL Buffer and 100% ethanol as indicated in Steps 7 and 9, Page 8.
Problem	Cause	Solution
Low A_{260}/A_{280} ratio	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.
	Poor cell lysis	Increase incubation time with CTL Buffer and Proteinase K Solution.

Ordering Information

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

Product	Part Number
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (100 mL)	PS010
RNase A (400 µL)	AC117