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Introduction

The EZgene™ Insect gDNA Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from insects, arthropods, and some plant tissue samples rich in polysaccharides. 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 ezBind™ 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 components of the EZgeneTM Insect gDNA Kit, except the Proteinase K and RNase A should be stored at 22°C-25°C. Once reconstituted in water, Proteinase K should be stored -20°C. Under at these conditions, DNA has successfully been purified and used for PCR after 12 months of storage. Store RNase A at 4 °C. All EZgene™ Insect gDNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at 22°C-25°C

Binding Capacity

Each ezBind™ DNA column can bind approximately 100 μg DNA. Using greater than 30 mg tissue is not recommended.

Kit content

Product	GD2413-00	GD2413-01	GD2413-02
Preps	4	50	250
ezBind DNA Columns	4	50	250
2 mL Collection tubes	8	100	500
Buffer ITL	2 mL	20 mL	100 mL
Buffer BL	2 mL	20 mL	100 mL
Buffer KB	2.8 mL	28 mL	135 mL
Proteinase K	2 mg	30 mg	5 x 30 mg
RNase A （20mg/mL）	25 μL	270 μL	1.35 mL
DNA Wash Buffer	2 mL	15 mL	3 x 24 mL
Elution Buffer	1 mL	15 mL	70 mL
User Manual	1	1	1

Before Starting

Please read the entire booklet to become familiar with the EZgene™ Insect gDNA Kit protocol.

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

GD2413-00	Add 8 mL absolute (96%-100%) ethanol.
GD2413-01	Add 60 mL absolute (96%-100%) ethanol to each bottle.
GD2413-02	Add 96 mL absolute (96%-100%) ethanol to each bottle.

Prepare proteinase K stock solution as following:

GD2413-00	Add 110 μL Elution Buffer to the vial
GD2413-01	Add 1.3 mL Elution Buffer to the vial
GD2413-02	Add 1.3 mL Elution Buffer to each vial

Insect gDNA Isolation Protocol

Materials to be provided by user

- Microcentrifuge capable of at least 14,000 x g
- Nuclease-free 1.5 mL or 2 mL microfuge tubes
- Water bath equilibrated to 65°C
- Equilibrate sterile ddH_2O or 10 mM Tris pH 8.5 at 65 °C
- Absolute (96%-100%) ethanol
- Chloroform and isoamyl alcohol

Insect 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.

Insects

1. Pulverize no more than **50 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 (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. Proceed to Step 2 below.

   Amount of starting material depends on 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 ezBind column as DNA 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 Buffer ITL** followed by **25 µL Proteinase K (25 mg/mL).** Vortex briefly to mix and incubate at 60°C for a minimum of 30 min 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 to mix. Centrifuge at 10,000 x g for 2 min 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 will remove much of the polysaccharides and proteins from solution and improve spin-column performance downstream. If there is very few upper aqueous phase present after centrifugation, add **200 µL** of **Buffer ITL** and vortex to mix. Centrifuge as above and transfer the upper aqueous phase to tube.

4. Add **1 volume** of **Buffer BL** followed by **5 µL RNase A**, vortex at maxi speed for 15 s. Incubate at 70°C for 10 min.

5. Add **1 volume** of **absolute ethanol** (room temperature, 96-100%) and mix well by vortexing at maxi speed for 15 s.

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

6. Apply **700 µL** of the mixture from step 5, including any precipitation that may have formed, to the ezBind DNA column. Centrifuge at 10,000 x g for 1 min at room temperature. Discard flow through liquid and re-use collection tube.

7. Place ezBind DNA column back into the same collection tube, apply the remaining of mixture into the column and centrifuge as above. Discard flow-through liquid and collection tube.

8. Place the column into another a new 2 mL collection tube (supplied) and wash by adding **500 µL Buffer**.**KB** Centrifuge at 10,000 x g for 30 s. Discard flow-through liquid and re-use collecting tube in next step.

9. Place column into the collection tube and wash by adding **650 µL DNA Wash Buffer** diluted with absolute ethanol. Centrifuge 10,000 x g for 30 s. 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 3. If refrigerated, the diluted DNA Wash Buffer must be brought to room temperature before use.

10. Repeat step 9 with a second **650 µL DNA Wash Buffer** diluted with ethanol. Discard liquid and collection tube. Insert the column to a new collection tube, with the lid open, and centrifuge the column at 13,000 x g for 2 min at room temperature.

    Note: This step is critical in removing traces of ethanol that will interfere with downstream applications.

11. Place column into a clean 1.5 mL microfuge tube (not suplied). To elute DNA add **50 µL-100 µL** of **Elution Buffer** (**10 mM Tris-HCL, pH 8.5**) preheated to

60°C-70°C directly onto the ezBind matrix. Allow soaking for 2 min at room temperature. Centrifuge at 13,000 x g for 1 min to Elute DNA.

12. Repeat elution step with a second **50 µL-100 µL Elution Buffer**.

Note: Typically a total of **5-15 µg DNA** with absorbance ratio (A_{260}/A_{280}) of 1.7-1.9 can be obtained. Yields vary depending on source and quantity of starting material used.

Note: To increase DNA Yield, add Elution buffer and incubate the column at 60°C-70°C for 5 min before elution.

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 A_{260}/ A_{280} 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 Absorbance$_{260}$ x {Dilution Factor}

Trouble Shooting Guide

Problem	Possible Cause	Suggestions
Clogged Column	Incomplete lysis	Increase incubation time with Buffer ITL / Proteinase K. An overnight incubation may be necessary.
	Sample too large	Do not use greater than 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 ℃ for 5 min before spin.
	Poor binding to column	Follow protocol closely when adjusting binding conditions.
	Improper washing	DNA Wash Buffer Concentrate must be diluted with ethanol before use.
Low 260A/A280 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 Buffer ITL. An overnight incubation may be necessary.
	Trace protein contaminants remain	Following step 8, wash column with a mixture of [300 μL Buffer BL + 300 μL ethanol] before proceeding to step 9.
No DNA eluted	Poor cell lysis.	Increase incubation time with Buffer ITL. 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 BL and absolute ethanol.
	No ethanol added to DNA Wash Buffer Concentrate	Dilute Wash Buffer with the indicated volume of absolute ethanol before first use.

Related EZgene™ Products

Catalog #	Product Name	Preps	Price $
GD2211-01	Tissue gDNA kit	50	90.00
GD2211-02	Tissue gDNA kit	250	420.00
GD2814-01	96-well tissue gDNA kit	4x96	680.00
GD2814-02	96-well tissue gDNA kit	20x96	3000.00
GD2311-01	Blood gDNA mini kit	50	90.00
GD2311-02	Blood gDNA mini kit	250	420.00
GD2312-01	Blood gDNA midi kit	10	90.00
GD2312-02	Blood gDNA midi kit	25	200.00
GD2314-01	Blood gDNA maxi kit	10	160.00
GD2314-02	Blood gDNA maxi kit	25	360.00
GD2815-01	96-well blood gDNA kit	4x96	680.00
GD2815-02	96-well blood gDNA kit	20x96	3000.00
GD2411-01	Bacterial gDNA kit	50	110.00
GD2411-02	Bacterial gDNA kit	250	495.00
GD2412-01	Soil gDNA kit	50	90.00
GD2412-02	Soil gDNA kit	250	420.00
GD2413-01	Insect gDNA kit	50	90.00
GD2413-02	Insect gDNA kit	250	420.00
GD2414-01	Mullusc gDNA kit	50	90.00
GD2414-02	Mullusc gDNA kit	250	420.00
GD2415-01	Yeast gDNA kit	50	90.00
GD2415-02	Yeast gDNA kit	250	420.00
GD2416-01	Fungal gDNA kit	50	90.00
GD2416-02	Fungal gDNA kit	250	420.00

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 Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga 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 technology support or learn more product information, please visit our website at www.biomiga.com or contact us at (858)603-3219.