Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Insect Genomic DNA Kit

rev 06/19

(Cat# K1412-50, -250; Ship at RT; Store at Multiple Temperatures)

I. **Introduction:**

BioVision's Insect Genomic DNA 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. Each ezBind DNA Column can bind approximately 100 µg DNA. Using >30 mg tissue is not recommended.

II. **Sample Types:**

- Insects, arthropods, and some plant tissue samples rich in polysaccharides

III. **Kit Contents:**

	K1412-50	K1412-250	
Components	**50 Preps**	**250 Preps**	**Part Number**
ezBind Columns	50	250	K1412-XX-1
Buffer MTL	20 mL	80 mL	K1412-XX-2
Buffer MBL	20 mL	80 mL	K1412-XX-3
Buffer KB	30 mL	120 mL	K1412-XX-4
Proteinase K	1.5 mL	6.0 mL	K1412-XX-5
RNase A	120 µL	500 µL	K1412-XX-6
DNA Wash Buffer*	12 mL	50 mL	K1412-XX-7
Elution Buffer	20 mL	60 mL	K1412-XX-8

Add 48 mL (K1412-50) or 200 mL (K1412-250) of (96%-100%) ethanol to each DNA Wash Buffer bottle (K1412-XX-7) before use.

IV. **User Supplied Reagents and Equipment:**

- Microcentrifuge capable of at least 12,000g
- Nuclease-free 1.5 mL or 2 mL microfuge tubes
- Water bath equilibrated to 65^{o}C
- Equilibrate Elution Buffer at 65^{o}C
- Absolute (96%-100%) ethanol
- Chloroform and isoamyl alcohol (24:1)

V. **Shipment and Storage:**

All components of the Insect Genomic DNA Kit, except the Proteinase K and RNase A should be stored at room temperature (RT). Once received, Proteinase K and RNAse A should be stored at -20°C. Insect Genomic DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored properly. DO NOT FREEZE!

VI. **Reagent Preparation and Storage Conditions:**

- 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.
- *Add 48 mL (K1412-50) or 200 mL (K1412-250) of (96%-100%) ethanol to each DNA Wash Buffer bottle ***(K1412-XX-7)*** before use.

VII. **Insects Protocol:**

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) Protocol:

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 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 MTL** followed by **25 µL Proteinase K** (20 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 hr. 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 10,000g 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 MTL** and vortex to mix. Centrifuge as above and transfer the upper aqueous phase to tube.*
4. Add **one volume of Buffer MBL** followed by 2 µL RNase A, vortex at maxi speed for 15 sec. Incubate at 70°C for 10 min.

5. Add **one volume of absolute ethanol** (RT, 96-100%) and mix well by vortexing at maxi speed for 15 sec. *Tips: 500 µL upper aqueous solution, add 500 µL Buffer BL 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 ezBind DNA column. Centrifuge at 10,000g for 1 min at RT. 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 10,000g for 30 sec. 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** diluted with absolute ethanol. Centrifuge at 10,000g for 30 sec. 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. If refrigerated, the diluted DNA wash buffer must be brought to RT before use.*
10. Repeat step 9 with another **600 µL DNA Wash Buffer**. Discard liquid and re-insert the column, with the lid open to the empty collecting tube.
11. Centrifuge the column at 12,000g for 2 min to remove the residual ethanol.
12. Place column into a clean 1.5 mL microfuge tube (not supplied). To elute DNA add 50-100 µL of Elution Buffer or ddH_2O preheated to 60-70°C directly onto the matrix. Allow soaking for 2 min at RT. Centrifuge at 10,000g for 1 min to Elute DNA.
13. Repeat elution step with a second 50 µl-100 µl Elution Buffer. Typically a total of 5-15 µg DNA with absorbance ratio (A260/A280) 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.*

VIII. 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}.

IX. Related Products:

Product Name	Cat. No.	Size
Bacterial Genomic DNA Isolation Kit	K309-100	100 Isolations
Bacterial Genomic DNA Mini Kit	K1388-100, -300	100 Preps, 300 Preps
Bacterial Genomic DNA Mini Kit (w/Lysozyme)	K1389-100, -300	100 Preps, 300 Preps
Concert™ DNA Extraction Kit	K1390-50, -100, -300	50 Preps, 100 Preps, 300 Preps
Genomic DNA Micro Kit	K1385-100, -300	100 Preps, 300 Preps
Genomic DNA Isolation Kit (Cells/Tissues)	K281-50	50 Assays
Genomic DNA Mini Kit (Blood/Cells)	K1380-100, -300	100 Preps, 300 Preps
Genomic DNA Maxi Kit (Blood/Cells)	K1381-10, -25	10 Preps, 25 Preps
Macro Blood DNA Kit	K1392-3, -1000	100 ml, 1000 ml
Macro Blood DNA Plus Kit	K1393-1000	1000 ml
Macro Bacterial DNA Kit	K1394-3, -1000	100 ml, 1000 ml
Macro Bacterial DNA Plus Kit	K1395-1000	1000 ml
Macro Tissue DNA Kit	K1396-3, -1000	100 ml, 1000 ml
Macro Tissue DNA Plus Kit	K1397-1000	1000 ml
Macro Cell DNA Kit	K1398-3, -1000	100 ml, 1000 ml
Macro Cell DNA Plus Kit	K1399-1000	1000 ml
Mammalian Cell Genomic DNA Isolation Kit	K967-100	100 Isolations
Mitochondrial DNA Isolation Kit	K280-50	50 Assays
Plant Genomic DNA Mini Kit	K1383-100	100 Preps
Plant Genomic DNA Maxi Kit	K1384-10, -25	10 Preps, 25 Preps
Plant Tissue Genomic DNA Isolation Kit	K316-100	100 Isolations
Swab Genomic DNA Kit	K1386-100, -300	100 Preps, 300 Preps
Tissue Genomic DNA Mini Kit	K1382-50, -100, -300	50 Preps, 100 Preps, 300 Preps
Triadzol™ Plant DNA Reagent	M1391-100, -200	100 ml, 200 ml
Yeast Genomic DNA Mini Kit	K1387-100, -300	100 Preps, 300 Preps
96 Well Blood Genomic DNA Kit	K1406-2, -4	2 Plates, 4 Plates
96 Well Plant Genomic DNA Kit	K1407-2, -4	2 Plates, 4 Plates
96 Well Plant Genomic DNA Binding Plate	M1209-5	5 Plates
Whole Blood DNA Isolation Kit	K528-100	100 Isolations

X. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Clogged Column	• Incomplete lysis • Sample too large • Incomplete homogenization	• Increase incubation time with Buffer ITL/Proteinase K. An overnight incubation may be necessary. Do not use greater than recommended amount of starting material. For larger samples, divide into multiple tubes. • Pulverize material as indicated in liquid nitrogen to obtain a fine powder.
Low DNA yield or no DNA eluted	• Clogged column • Poor elution • Poor binding to column • Improper washing	• As above • Repeat elution or increase elution volume. Incubate the column at 70℃ for 5 min before spin • Follow protocol closely when adjusting binding conditions. • DNA Wash Buffer Concentrate must be diluted with ethanol before use.
Low A_{260}/A_{280} ratio	• Extended centrifugation during elution step • Poor cell lysis • Trace protein contaminants remain	• 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. • Increase incubation time with Buffer ITL. An overnight incubation may be necessary • Wash column with a mixture of [300 µL Buffer MBL + 300 µL ethanol] before proceeding to step 9.

FOR RESEARCH USE ONLY! Not to be used on humans.