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# Mollusc Genomic DNA Kit

**(Cat# K1413-50, -250; Ship at RT; Store at Multiple Temperatures)**

## I. Introduction:

**BioVision's Mollusc Genomic DNA Kit** is designed to extract 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. Samples are homogenized and lysed in a high salt buffer and extracted with chloroform to remove mucopolysaccharides. Following a rapid alcohol precipitation step, binding conditions are adjusted and DNA further purified using ezBind DNA spin columns. While proteins and other contaminants are removed by wash buffer, high quality genomic DNA is eluted with elution buffer or sterile water. The purified genomic DNA is suitable for downstream applications such as Southern Blot, restriction digestion, and PCR. Each ezBind DNA column can bind approximately 100 µg DNA.

## II. Sample Types:

- Molluscs, insects, arthropods, roundworms, flatworms, and other invertebrates rich in mucopolysaccharides.

## III. Kit Contents:

| Components | K1413-50<br>50 Preps | K1413-250<br>250 Preps | Part Number |
|---|---|---|---|
| ezBind DNA Columns | 50 | 250 | K1413-XX-1 |
| Buffer MTL | 20 mL | 80 mL | K1413-XX-2 |
| Buffer MBL | 20 mL | 80 mL | K1413-XX-3 |
| Buffer KB | 28 mL | 120 mL | K1413-XX-4 |
| Proteinase K | 1.3 mL | 6 mL | K1413-XX-5 |
| RNase A | 120 µL | 500 µL | K1413-XX-6 |
| DNA Wash Buffer* | 15 mL | 50 mL | K1413-XX-7 |
| Elution Buffer | 15 mL | 60 mL | K1413-XX-8 |

**Add 48 mL (K1413-50) or 200 mL (K1413-250) 96%-100% ethanol to each DNA Wash Buffer bottle before use.*

## IV. User Supplied Reagents and Equipment:

- Microcentrifuge capable of at least 13,000g; Nuclease-free 1.5 mL or 2 mL microfuge tubes
- Water bath or heating block preset to 65°C
- Equilibrate Elution Buffer at 65°C
- Absolute (96%-100%) ethanol; Chloroform and isoamyl alcohol (24:1)

## V. Shipment and Storage:

All components of the Mollusc 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. During shipment, or storage in cool ambient conditions, precipitates may form in some buffers. It is possible to dissolve such deposits by incubation the solution at 65°C. All Mollusc 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:

- 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. 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 DNA 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.
- Add 1.3 mL Elution Buffer to the vial Proteinase K for either (K1413-50) or (K1413-250).
- Add 60 mL (K1413-50) or 96 mL (K1413-250) to each DNA Wash Buffer bottle before use.

## VII. Molluscs (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.

**Arthropods Protocol:**

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.
2. Add 350 µL Buffer MTL followed by 25 µL Proteinase K. Vortex to mix and incubate at 60°C for a minimum of 30 min or until entire sample is solubilized. Actual incubation time varies and depends on elasticity of tissue. 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 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 very few upper aqueous phase present after centrifugation, add 200 µL of MTL Buffer 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 (room temperature, , 96-100%) and mix well by vortexing at maxi speed for 15 sec. *Note: 500 µL upper aqueous solution, add 500 µL Buffer MBL and 500 µL of absolute ethanol.*
6. Apply 750 µL of the mixture from step 5, including any precipitation that may have formed, to an ezBind DNA column Centrifuge at 10,000g 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 10,000g for 30 sec. Discard the flow-through and re-use collection tube.
9. Place column into collection tube from previous step and add 600 µL DNA Wash Buffer diluted with ethanol. Centrifuge 10,000g 1 min as above. Discard flow-through liquid and re-use collecting tube in next step. *Note: That DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle and page 4. If refrigerated, the diluted DNA wash buffer must be brought to room temperature before use.*
10. Repeat step 9 with a second 600 µL DNA Wash Buffer diluted with ethanol. Discard liquid and collection tube. And insert the column into a new collecting tube
11. Centrifuge the column at 12,000g for 2 min to remove the residual ethanol. This step is critical in removing traces of ethanol that will interfere with downstream applications.
12. Place column into a clean 1.5 mL microfuge tube (not supplied). To elute DNA add 50 -100 µL of Elution Buffer or dd H2O preheated to 60-70°C directly onto the matrix. Allow soaking for 2 min at room temperature. Centrifuge at 10,000 x g for 1 min to Elute DNA.
13. Repeat elution step with a second 50 ul-100 ul 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 dd$H_2O$ 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. General Troubleshooting Guide:

| Problems | Possible Reasons | Solutions |
|---|---|---|
| Clogged Column | • Incomplete lysis<br>• Sample too large<br>• 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.<br>• Pulverize material as indicated in liquid nitrogen to obtain a fine powder. |
| Low DNA yield or no DNA eluted | • Clogged column<br>• Poor elution<br>• Poor binding to column<br>• Improper washing | • As above.<br>• Repeat elution or increase elution volume. Incubate the column at 70°C for 5 min before spin<br>• Follow protocol closely when adjusting binding conditions.<br>• DNA Wash Buffer Concentrate must be diluted with ethanol before use. |
| Low 260A/A280 ratio | • Extended centrifugation during elution step<br>• Poor cell lysis<br>• 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.<br>• Increase incubation time with Buffer ITL. An overnight incubation may be necessary<br>• 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<br>• Incomplete homogenization<br>• Absolute ethanol not added before adding sample to the column<br>• No ethanol added to DNA Wash Buffer Concentrate | • Increase incubation time with Buffer ITL. An overnight incubation may be necessary.<br>• Pulverize starting material as indicated in liquid nitrogen to obtain a fine powder<br>• Before applying DNA sample to column, add Buffer BL and absolute ethanol.<br>• Dilute Wash Buffer with the indicated volume of absolute ethanol before first use. |

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