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Plant Tissue Genomic DNA Isolation Kit

rev 03/21

(Catalog # K316-100; 100 isolations; Store at -20 °C/RT)

I. Introduction:

Plants serve a vital role in human nutrition, biofuels, and the ecosystem. Isolating DNA from plants is exceptionally challenging compared to other organisms due to the presence of difficult to disrupt cell wall and compounds such as tannins, phenolics, and complex polysaccharides that can affect DNA quality and inhibit downstream reactions. **BioVision's Plant Genomic DNA Isolation Kit** enables the researchers to isolate genomic DNA from plant leaves, stalks, seeds, and roots. This kit utilizes an efficient combination of lysis buffer and enzymes that work in unison to disrupt the cell wall, eliminate plant specific contaminating compounds, and release free genomic DNA. DNA release from the cell is coupled with adsorption of DNA onto a silica spin-column under chaotropic conditions, eliminating the use of toxic organic compounds or solvents. This kit yields pure DNA that is suitable for various downstream molecular biology applications such as PCR, cloning, sequencing, DNA hybridization and Southern Blotting, etc.

II. Applications:

- PCR and cloning
- Sequencing
- DNA Hybridization
- Southern Blotting

III. Sample Types:

- Fresh or dry leaf, stalk, root, and seed plant tissue.

IV. Kit Contents:

Components	K316-100	Cap Code	Part Number	Storage (°C)
Buffer L [Lysis Buffer]	40 ml	Amber/NM	K316-100-1	RT
1M DTT	400 µl	Green	K316-100-2	-20 °C
Enzyme Mix	2 x 1.0 ml	Red	K316-100-3	-20 °C
RNAse A	2 x 1.0 ml	Blue	K316-100-4	-20 °C
Buffer B [Binding Buffer]	25 ml	NM	K316-100-5	RT
Buffer W [Wash Buffer]	30 ml	WM	K316-100-6	RT
Buffer E [Elution Buffer]	22 ml	WM	K316-100-7	RT
Spin Columns/Collection Tubes	100 tubes	-	K316-100-8	RT

V. User Supplied Reagents and Equipment:

- DNAse-free aerosol tips and micro-centrifuge tubes
- Ethanol
- Heating Block
- Centrifuge

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C and room temperature (RT), protected from light. Briefly centrifuge all small vials prior to opening. Read entire protocol before performing the assay. Use DNAse-free tubes and DANse-free aerosol tips at all times.

- **Buffer L:** Store at room temperature (RT) and reconstitute a desired amount with 10mM DTT immediately before use (e.g. add 4µL of 1M DTT to 396µL of Buffer L). ***Note:*** *Do not store unused Buffer L reconstituted with DTT.*
- **Buffer B:** Add 28 mL of 100% Ethanol, molecular biology grade. Mix well and store at RT.
- **Buffer W:** Add 136 mL of 100% Ethanol, molecular biology grade. Mix well and store at RT.
- **Buffer E:** Ready to use. Store at RT.
- **Enzyme Mix** and **RNAseA:** Ready to use. Store at -20 °C. Keep on ice at all times while in use.
- **Spin Columns:** Ready to use. Store at RT in dry conditions.

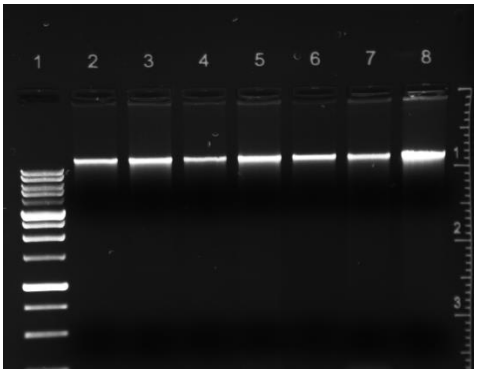


Figure 1. Example genomic DNA isolation using BioVision's Plant Genomic DNA Isolation Kit. Lane 1 – BriteRuler 1kb DNA ladder; 2 – Cucumber Leaf gDNA; 3 – Bell Pepper Leaf gDNA; 4 – Tomato Leaf gDNA; 5 – Green Bean Leaf gDNA; 6 – Potato Root gDNA; 7 – Corn Seed gDNA; 8 – Pea Seed gDNA.

VII. Bacterial Genomic DNA Extraction Protocol:

1. Sample Preparation:

a. Weigh out 20-30 mg of dry plant tissue. If using fresh tissue, weight out 50-100 mg of plant material and grind it to fine powder in liquid nitrogen using a mortar and pestle or use other plant cell disruption method of your choice such as bead beating.

Note: If using >100mg of fresh tissue or >30 mg of dry tissue, scale up the entire protocol proportionally.

b. Transfer the powder to a clean 1.5 mL conical tube and immediately add 400 µL Buffer L [Lysis Buffer] reconstituted with 10 mM DTT to disrupted plant tissue and vortex for 2-3 sec to re-suspend.

2. DNA Release:

c. Add 20 µL of Enzyme Mix. If RNA free DNA is desired, add 20 µL of RNAse A solution at this time. Mix by inverting the tube 3-5 times.

d. Incubate for 1 hour at 55 °C, then increase the temperature to 65 °C and incubate for 20 more min. Tap the tube every 10-15 min to mix throughout the incubation period.

e. Centrifuge the tubes at 12,000 x g for 5 min at 4 °C.

f. Transfer 250 µL the clarified supernatant into a new clean 1.5mL micro-centrifuge tube. Avoid transferring cell debris.

3. Binding:

g. Add 500 µl of Buffer B [Binding Buffer] to each tube and mix by pipetting up and down until the solution becomes clear.

h. Place the provided spin column into the provided collection tube and pipette the entire supernatant onto the top of the column.

i. Centrifuge the spin column at 12,000 x g for 1 min at 4 °C and discard the flow through.

4. Washing:

j. Add 750 µl of Buffer W [Wash Buffer] onto the top of the spin column and centrifuge (12,000 x g; 1 min, 4 °C). Discard the flow through. Repeat this step one more time.

k. Centrifuge the spin column at 12,000 x g for 2 min at 4 °C to dry.

5. Elution:

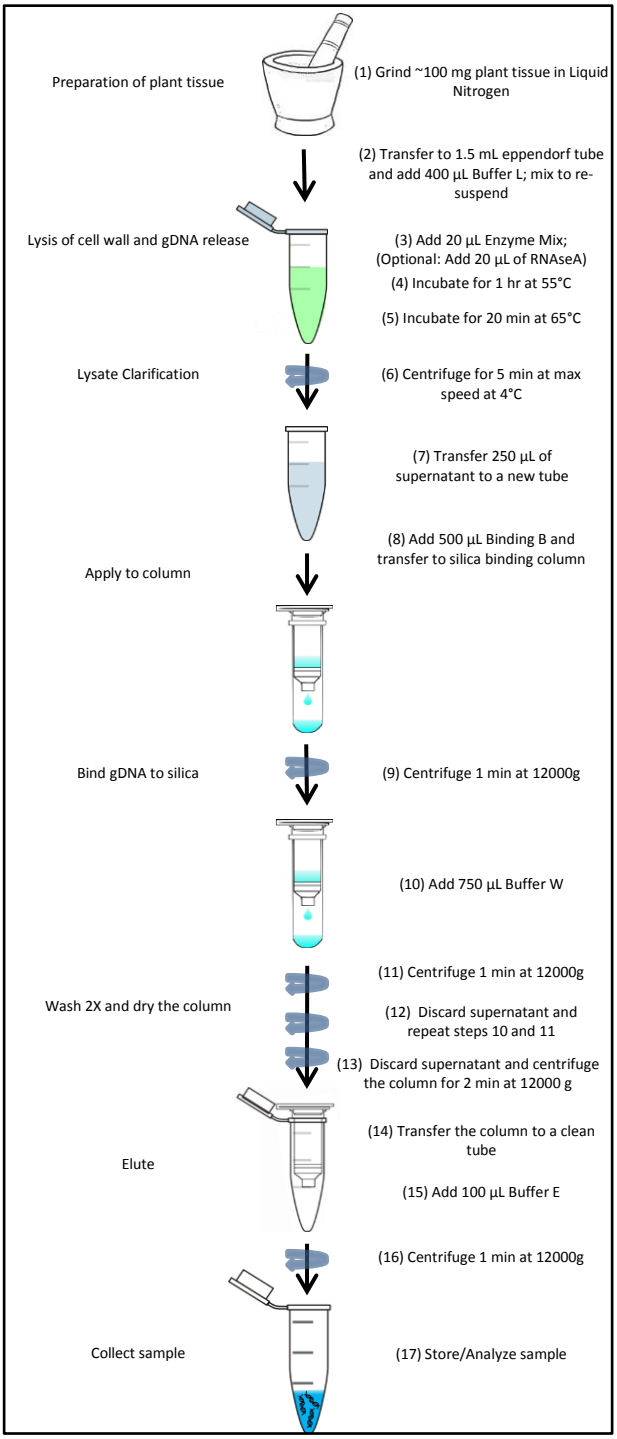
l. Transfer the spin column to a clean, DNAse-free 1.5mL tube.

m. Add 100 µl of Buffer E [Elution Buffer] to the top of the spin column and incubate for 1-2 min at room temperature.

j. Centrifuge at 12,000 x g for 1 min at 4 °C to elute the genomic DNA. The flow through contains purified DNA.

k. Sample is now purified and ready to use. Store genomic DNA at -20 °C or immediately use the sample in a downstream application of your choice.

Note: Generally, good quality genomic DNA will have A260/280 of 1.6 - 1.99 and exhibit one clear band of high molecular weight on 1% agarose gel. See troubleshooting guide in Section VIII for help.



Quick Protocol Guide

VIII. Troubleshooting:

Issue	Possible Reason	Recommendations
Low yield	Incompletely disrupted plant cells	Make sure to grind plant tissue in liquid nitrogen until it reaches a fine powder consistency.
	Incomplete Lysis/ DNA Release	Increase incubation time in Buffer L + Enzyme Mix at 55°C. Incubation time may be different for each plant species.
Low A260/280 (<1.6)	Protein Contamination	Increase incubation time in Buffer L + Enzyme Mix at 55°C.
High A260/280 (>2.0)	RNA Contamination	Add RNAse A during the cell lysis step.
No DNA band/severe smear on gel	DNAse contamination	Use DNAse free aerosol tips, DNAse-free tubes, and practice good sterile technique.

FOR RESEARCH USE ONLY! Not to be used on humans