

# Plant Genomic DNA Fast Isolation Kit

## (Spin-column)

*A fast kit for the isolation of genomic DNA from plant*

For laboratory research use only

DP3111 50 preps

### Kit Content, Storage, and Stability

Content	Storage	50 preps
Buffer P1	RT	30 ml
Buffer P2	RT	7 ml
Buffer P3	RT	25 ml
RNase A	-20°C	200 ul
Buffer WB	RT	15 ml
	Add ration ethanol before first using	
Buffer EB	RT	15 ml
Separation Column A	RT	50
Spin Column AC	RT	50
Collection Tube (2 ml)	RT	50

All reagents are stable for 12 months when stored properly.

**Dilute Buffer WB with four volumes of absolute ethanol before starting and mark the label on the bottle.**

Buffer P1 and Buffer P2 may form precipitates due to low storage temperature. If necessary, dissolve the buffer by warming to 37°C until become clear. Cool to the RT before use.

Keep bottles with buffers tightly capped when not in use, preventing reagents evaporating, oxidation and pH changing.

### Principle

Dry or fresh plant tissue are disrupted and then lysed in a special buffer containing detergent. Proteins, polysaccharides and cellular debris are subsequently precipitated. Contaminants are further removed by isopropanol precipitation of DNA. Binding conditions are then adjusted. The sample is applied to a spin column and centrifuged. DNA binds to the silica membrane while contaminants such as proteins and polysaccharides are efficiently removed by two steps. Purified DNA is eluted in a small volume of low ion strength or water.

### Features

1. Fast, simple, the single sample can be finished under 60 min.
2. Stable, high quality silica membrane and ideal buffer system ensure the reproducible results.
3. High purity, the value of OD260/OD280 achieves 1.7-1.9, which can be applied to PCR, Southern-blot and digestions directly.
4. Isolates DNA ranges from 30 kb to 50 kb.

### Notes

**Please read this section before your experiment**

1. All the centrifugation steps can be performed at room temperature.
2. Prepare the beta-mercaptoethanol by yourself.
3. Buffer P3 includes the stimulating compound. Please wear latex gloves, avoiding skin, eyes and cloth to be contaminated. If that, use water of physiological saline washing.
4. EDTA-free buffer EB – no influence on down-stream reactions. For elution you can use water, but the pH has to be more than 7.5. Store DNA at -20°C.

## Procedure

**Dilute buffer WB with four volumes of absolute ethanol before starting and mark the label.**

**Pre-warm Buffer P1 to 65°C and add beta-mercaptoethanol to the final concentration 0,2%.**

1. Pre-warm buffer P1, mortars, pestles and sterilized water to 65°C.
2. Take proper plant tissue to mortar and make it into powder with liquid nitrogen
3. Transfer powder (fresh plant tissue 100 mg, gross weight tissue 30 mg) to a 1,5 ml microtube and add 550 ul pre-warm buffer P1 and 4 ul RNase A. Vortex 1 min and let it at room temperature for 10 min.
4. Add 130 ul of Buffer P2 and mix thoroughly. Centrifuge at 12.000 rpm for 3 min.
5. Carefully transfer the supernatant to a **Separation column**. Centrifuge at 12.000 rpm for 1 min. **Collect the flow through (it contains DNA!).**
6. Add 1,5 volume of buffer P3 to the flow through and mix well.
7. Place a Spin column AC to the collection tube. Add the mixture (including precipitate!) to the spin column AC. Centrifuge at 12.000 rpm for 1 min. Discard the flow-through.
8. Add 700 ul of Buffer WB and centrifuge at 13.000 rpm for 1 min. Discard the flow-through.  
**NOTE: Buffer WB must be diluted with 100% ethanol before use.**
9. Add 500 ul of Buffer WB. Centrifuge at 13.000 rpm for 1 min. Discard the flow-through.
10. Centrifuge the empty column at 13.000 rpm for 3-5 min.
11. Transfer the column AC to a clean 1,5 ml microtube, add 50 ul of Buffer EB, pre-warmed to 65-70°C, directly onto the silica membrane. Incubate 3-5 min at room temperature. Centrifuge at 13.000 rpm for 1 min.

**The volume of elution buffer can be adjusted according to needs. Reduced elution volume can increase final DNA concentration, but the minimum elution volume is 50 ul. Too low elution volume (under 50 ul) decreases the elution efficiency and the final DNA yield.**

12. Keep DNA at 2-8°C, for long term storage at -20°C.

## Troubleshooting:

Problem	Possible causes	Advice
Low DNA yield	Sample excess or incomplete lysis	Use proper amount of sample and sufficiently grinded
RNA contamination	RNA rich plant	Add 8 ul RNase A instead of 4 ul.
No product	No ethanol in Buffer WB	Add 100% ethanol before starting
DNA colored	Not enough wash times Too much material	Add 500 ul buffer WB or 100% ethanol to the wash after step 7. Reduce material, do not exceed.
Low DNA elution	Ethanol residues in spin column or collection tube bottom Use water or other solution, replace buffer EB	Ensure step 10, as it affect the elution efficiency. Read carefully step 10, just use buffer EB.
A260 too high	Silicified membrane eluted, influence A260 value	Centrifuge at 13.000 rpm for 1 min, carefully using the supernatant.
DNA digestion inhibition	Silicified membrane eluted, inhibited digestion Ethanol residues in spin column or collection tube bottom	Centrifuge at 13.000 rpm for 1 min, carefully using the supernatant. Ensure do step 10 and air dry for a moment.