Note: for laboratory research use only

Plant DNA Rapid Extraction Kit
(Spin-column)

Cat. #: DP3111 (50 preps)
DP3112 (100 preps)

Signalway Biotechnology

Ⅰ. Kit Content, Storage and Stability

Content	Storage	50 preps （DP3111）	100 preps （DP3112）
Buffer P1	RT	30 ml	60 ml
Buffer P2	RT	7 ml	14 ml
Buffer P3	RT	50 ml	100 ml
RNase A	-20℃	200 μl	400 μl
Buffer EB	RT	15 ml	20 ml
Buffer WB	RT	15 ml	25 ml
		Add 60 ml ethanol before first use	***Add 100 ml ethanol before first use***
Separation Column A	RT	50	100
Spin-column AC	RT	50	100
Collection Tube (2ml)	RT	50	100

All reagents are stable for 12 months if stored properly.

Reminder:

1・***Add ethanol to Buffer WB before use, mix adequately, and then check the box on the label showing ethanol was added!***

2・Buffer P1 and P3 may precipitate under low temperature. Incubate at 37°C for a few minutes **until buffer is clear**, and then let it cool to RT before use.

3・Keep reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

Ⅱ.Principle:

Dry or fresh plant tissues are grinded and then lysed in a special buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated. Binding conditions are optimized. The sample is then applied to a spin-column and centrifuged. DNA binds to the silicified membrane while contaminants such as

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proteins and polysaccharides are efficiently removed by two-step wash. Purified DNA is eluted in a small volume of low ionic strength buffer or DNase-free water.

Ⅲ. Features:

1 · The kit does not contain poisonous phenol and does not need a step of ethanol precipitation. Multi-elution can ensure high-purified DNA, 30 Kb to 50 Kb, which can be applied to all kinds of molecular biology experiments such as PCR, Southern-blot, restriction enzyme digests, and mammalian transfections.

2 · Stable and high-quality silicified membrane and ideal buffer system ensure the reproducible results.

3 · The yield of DNA is excellent.

Ⅳ. Notes

Read this section before your experiment.

1. All the centrifugation can be performed at room temperature.
2. Buffers P3 contains a corrosive compound; please wear latex gloves to avoid contact with skin, eyes, and clothes**. If contact occurs, wash with water or physiological saline.**
3. DNA typically has an A260/A280 ratio between 1.7 and 1.9.
4. There is no EDTA in Buffer EB, which should not affect down-stream reactions.

   If interference occurs, use sterile water (pH >7.5) to elute and store DNA at -20℃.

   Low pH will decrease the elution efficiency. For long-term storage, elute DNA in TE (10mM Tris-HCl, 1mM EDTA, pH 8.0) and be sure to dilute the DNA solution before use because the EDTA will affect down-stream reactions.
5. Set water bath to 60-70°C.
6. β-Mercaptoethanol should be prepared by the user.

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Ⅴ. Procedure

Before Starting

- ***Dilute Buffer WB with absolute ethanol, vortex adequately, then mark the check box!***
- ***Pre-warm Buffer P1 to 65°C and add β-mercaptoethanol at the final concentration 0.2%.***

1. Pre-warm buffer P1, mortars, pestles, and sterilized water to 65℃
2. Take proper plant tissue to mortar and grind in liquid nitrogen.
3. Transfer powders (fresh plant tissue 100 mg or gross weight tissue 30 mg) to a 1.5 ml centrifuge tube and add 550 µl pre-warm Buffer P1 (added β-mercaptoethanol to final concentration 0.2%) and 4 µl RNaseA. Vortex for 1 min and let it cool down to room temperature for 10 minutes.
4. Add 130 µl Buffer P2 and mix thoroughly. Centrifuge at 12,000 rpm for 3 minutes.
5. Carefully transfer the supernatant to a Separation column A, centrifuge at 12,000 rpm for 1 minute, and collect the flow-through.
6. Add 1.5 volumes of Buffer P3 to the flow through and mix thoroughly.
7. Place a Spin-column AC to a collection tube. Transfer the mixture (including precipitate) to the Spin-column AC. Centrifuge at 12,000 rpm for 1 minute. Discard the flow through.
8. Add 700 µl Buffer WB (**check if ethanol is added!**). Centrifuge for 1 minute at 13,000 rpm. Discard the flow-through.
9. Add 500 µl buffer WB. Centrifuge for 1 minute at 13,000 rpm. Discard the flow-through.
10. Then centrifuge the empty Spin-column AC at 13,000 rpm for 3-5 minutes.
11. Transfer the Spin-column AC to a clean 1.5 ml microcentrifuge tube, add 50 µl Buffer EB (warm in 65-70℃ before use) directly onto the silicified membrane. Incubate 3-5 minutes at room temperature. Centrifuge at 13,000 rpm at 1 minute.

    The volume of elution buffer could be adjusted according to needs. Appropriate reduction of elution volume can increase concentration. However, the minimum

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volume is 50 µl. If the elution volume is less than 50 µl, elution efficiency and DNA yield can be affected.

12. Keep DNA at -20℃ or apply to down-stream reactions

Ⅶ. Troubleshooting

Problem	**Possible causes**	**Advices**
Low DNA yield	Excessive sample or incomplete lysis	Use proper amount of sample, which should be completely grinded.
RNA contamination	RNA rich in plant	Add 8 µl RNase instead 4 µl in step 3
No Product	Not add ethanol to Buffer WB	Add the ethanol before use.
DNA colored	Not enough wash times	Add 500 µl WB or 100% ethanol to wash after step 7
	Too much sample	Reduce material
Low DNA elution	Ethanol residues in spin column or collection tube bottom.	Ensure that step 10 is performed
	Use water or other solution rather than buffer EB	Please reading carefully step 11, just use Buffer EB
A_{260} too high	Silicified membrane eluted, which influences A_{260} value.	Centrifuge at 13,000 rpm for 1 min, save the supernatant.
DNA digestion inhibition	Silicified membrane eluted, which inhibits digestion.	Centrifuge at 13,000 rpm for 1 minutes, save the supernatant.
	Ethanol residues in Spin-column or collection tube bottom.	Ensure that step 10 is performed; air dry for a moment.

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