Transiom™ Plant gDNA Extraction kit

Cat No: TPDK-50/100/200

Contents

Introduction

The Plant DNA Kit provides a fast and simple method to isolating gDNA from Plant tissues. In the process, sample crushed with liquid nitrogen and lysed further with Lysis buffer (TPL). In the presence of binding buffer with chaotropic salt, the genomic DNA in the lysate binds to the silica column. The DNA is bound to the column while proteins and other impurities are removed by wash buffer.

Quality Control

Plant gDNA kit was tested by isolating of genomic DNA from 50 mg young and dry leaves. The purified DNA was quantified with Nanodrop 8000 and yield of genomic DNA was up to the 10 µg with the OD260/OD280 ratio of 1.8-2.0 .However, due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to≤100 mg. The yield of DNA varies from plant to plant or depends on the nature of tissues. Best results are obtained with young leaves.

Materials supplied by user:

- **Sterile pestle & mortar**
- **Nuclease-free microfuge tubes**
- **Liquid nitrogen**
- **Water bath Equilibrated to 65ºC.**
- **Nuclease free ddH_2O**
- **Absolute (96%-100) ethanol**
- **β-mercaptoethanol.**
- **Chloroform/Isoamyl alcohol (24:1)**

Kit Contents

Product	TPDK-50	TPDK-100	TPDK-200	Storage
Buffer TPL	48.0 ml	96.0 ml	192.0 ml	RT
Buffer TPB	36.0 ml	72.0 ml	144.0 ml	RT
Buffer TWI	15.0 ml	30.0 ml	60.0 ml	RT
TransPure™ High Binding Spin Columns	50 nos	100 nos	200 nos	RT
RNAse A	11 mg	22 mg	22 mg × 02	-20^{o}C
Elution Buffer	10 ml	15 ml	30 ml	RT
User Manual	1	1	1	

Before Starting

- Read carefully all manual instructions before starting
- RNAse A should be store at -20°C

Important:

For 50 Extractions

☑ Dilute **Buffer TW I** with **absolute ethanol** as follows and store at room temperature:

Buffer TW I: Add **60 ml** absolute (99%-100%) ethanol per bottle

☑ Add **550µl Elution Buffer** in **RNase A** containing vial. (Store at -20°C after reconstitute)

For 100 Extractions

☑ Dilute **Buffer TW I** with **absolute ethanol** as follows and store at room temperature:

Buffer TW I: Add **120 ml** absolute (99%-100%) ethanol per bottle

☑ Add **1100µl Elution Buffer** in **RNase A** containing vial. (Store at -20°C after reconstitute)

For 200 Extractions

☑ Dilute **Buffer TW I** with **absolute ethanol** as follows and store at room temperature:

Buffer TW I: Add **240 ml** absolute (99%-100%) ethanol per bottle

☑ Add **1100µl Elution Buffer** in **RNase A** containing vial. **(× 2 Vials)** (Store at -20°C after reconstitute)

Plant gDNA Kit Protocol

Step 1: Plant tissue selection and crushing.

1. Take 50-100 mg of fresh or stored plant tissues and grind in liquid nitrogen to make fine powder using mortar and pestle.
2. Transfer it into a microfuge tube (Not provided)

Step 2: Lysis

3. Add 800 µl of Buffer TPL with 15µl β-mercaptoethanol (Not provided) to the tube and mix it well. Add 10 µl of RNAse A into the lysate before incubation to remove the RNA.
Note: Dissolve RNAse A with elution buffer (refer to before starting note).

4. Incubate tissue lysate result from above step at 65 °C for 60 min. Mix sample during incubation by inverting tube.
Note: Increase Incubation time up to 120 – 180 min as per sample type.

5. Add 700 µl Chloroform/ Isoamyl alcohol (24:1) to the same microfuge tube and vortex to mix. Centrifuge at 12,000 rpm for 5-10 min.

6. Carefully aspirate supernatant resulting supernatant to a new 2 mL microfuge tube, make sure not to disturb the pellet or transfer any debris.

Step 3: Binding of DNA

7. Add 600 µl of Buffer TPB to the resultant supernatant from step 6 and mix well with inverting tubes 20-25 times.

8. Transfer 650 µl lysate to the DNA spin column, and centrifuge at 12,000 rpm for 2 min. Discard the flow through liquid.

9. Repeat above step, until the entire sample has been processed and retain column for further processing.

Step 4: DNA Washing

10. Place the column into same collection tube. Add 650 µl of Buffer TWI. Centrifuge at 12,000 rpm for 1 min. Discard the flow through.

11. Repeat the above "Step-10"
Note: Buffer TWI must be diluted with absolute (96%-100%) ethanol prior to use (refer to before starting note).

12. Place empty DNA spin column, into the same collection tube and centrifuge at 12,000 rpm for 2 min.

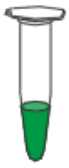
Step 5: DNA Elution

13. Place the column into a new sterile 1.5 ml eppendorf tube, add 30 µl preheated Elution buffer. Incubate at room temperature for 5 min. (perform this step twice 30 µl + 30 µl = 60 µl)

14. Centrifuge 12,000 rpm for 1 min to elute pure Plant gDNA. The first elution normally yields 60-70 % of DNA bound. A second elution with another 30 µl buffer will yield another 20 % of the DNA.
Note: Elution volume may vary as per downstream process

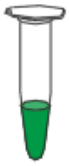
14. Discard the Column, and save elute. Do not reuse binding columns or collection tubes.

Flow Chart



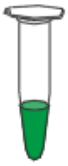
Preparation of samples

- Take 50-100 mg plant sample and grind it with LN2



Lysis

- Add 800 µl of Buffer TPL with 15 µl β-mercaptoethanol

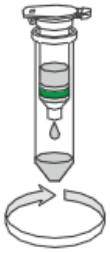


Incubation & Phase Separation

- Incubate at 65^{0}C for 60 min
- Add Chloroform / Isoamyl alcohol

Binding to silica column

- Adjust binding of phase with Buffer TPB



Washing

- Washing twice with wash buffer

Elution of Pure DNA

Hand on time

Trouble Shooting Guide

Problems	Possible reason	Suggestions
Clogged column	Incomplete Lysis	Add the correct volume of Buffer TPL and incubate for specified time at 65°C. It may be necessary to extend incubation time by 10 min.
	Sample is too high	If using more Plant Tissue sample increase volume of β-mercaptoethanol, Buffer TPL, Buffer TPB. Pass aliquots of lysate through one column successively.
Low DNA Yield	Clogged Column	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 70°C for 5 min with elution buffer may increase yields.
	Improper washing	TWI buffer concentrate must be diluted with ethanol.
Low A260/280 Ratio	Extended centrifugation during elution	Resin from the column may be present in elute and effect the OD absorbance. Avoid centrifugation at speed higher than 12,000 rpm.
	Poor cell lysis due to incomplete mixing with buffer TBA	Repeat the procedure, this time making sure to vortex the sample with Buffer TPL immediately and completely.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer TBA	Mix thoroughly with buffer TPL.

Limited Use and Warranty

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