Genomic DNA Isolation Kit (Plant)

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For research use only

Sample: up to 100 mg of fresh plant tissue or 50 mg of dry plant tissue

Format: spin column

Operation time: within 60 minutes

Introduction

The Genomic DNA Isolation Kit (Plant) was designed specifically for genomic DNA isolation from Plant samples. This unique buffer system ensures total DNA with high yield and good quality from samples and the spin column system was designed to purify or concentrate DNA products which have been previously isolated using buffers. The entire procedure can be completed in 1 hour without phenol / chloroform extraction. Purified DNA is suitable for use in PCR or other enzymatic reactions.

Kit Contents

Catalog No.	PC0004	PC0100	PC0200	PC0300
PL Buffer	2 ml	55 ml	105 ml	125 ml+30 ml
W1 Buffer	2 ml	45 ml	85 ml	125 ml
W2 Buffer (Add Ethanol)	300µl×2 (1.2 ml)×2	15 ml (60 ml)	25 ml (100 ml)	25 ml X 2 (100ml) X 2
EL Buffer	1 ml	10 ml	20 ml	30 ml
PC Columns	4 pcs	100 pcs	200 pcs	300 pcs
Collection Tubes	4 pcs	100 pcs	200 pcs	300 pcs

Quality Control

In accordance with FairBiotech's ISO-certified Total Quality Management System, the quality of the Genomic DNA Isolation Kit (Plant) is tested on a lot-to-lot basis to ensure consistent product quality.

Additional requirements

RNase A (50 mg/ml), Isopropanol, absolute ethanol, mortar and pestle, microcentrifuge tubes.

NOTE

- ★ Add ethanol (96–100%) to Buffer W2, **shake before use** (see bottle label for volume).
- ★ Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
- ★ Buffers W1 contain irritants. Wear gloves when handling these buffers.

Protocol

Step 1 Sample Preparation

- Cut off 50 mg of fresh plant tissue or 25 mg of dry plant tissue. Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.

Step 2 Lysis

- Add **500 µl of PL reagent and 0.5 µl of RNase A (50 mg/ml)** to the sample in the mortar and grind the sample until it is completely dissolved.
- Transfer the dissolved sample to a 1.5 ml microcentrifuge tube. Incubate at 75ºC for 30 minutes (invert the tube every 10 minutes).
- Centrifuge at 14-16,000 x g for 5 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

#Pre-heat the Elution Buffer to 75ºC for Step 6 DNA Elution.

Step 3 DNA Binding

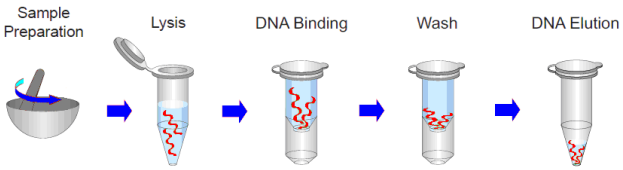
- **Add the same volume of Isopropanol** to the clear supernatant from the previous step and vortex immediately for 5 seconds (eg. add 350 µl Isopropanol to 350 µl supernatant)
- Place a **PC Column** in a **Collection Tube**. **Transfer the mixture** to the **PC Column**.
- Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the **PC Column** back in the **Collection Tube**.

Step 4 Wash

- Add **400 µl of W1 Buffer** into the **PC Column**. Centrifuge at 14,000 x g for 30 seconds.
- Discard the flow-through and place the **PC Column** back into the same **Collection tube**.
- Add **600 µl of W2 Buffer** (Ethanol added) into the **PC Column**. Centrifuge at 14,000 x g for 30 seconds.
- Discard the flow-through and place the **PC Column** back into the same **Collection tube.**
- Centrifuge at 14,000 x g again for 2 minutes to remove residual **W2 Buffer**.

Step 5 DNA Elution

- Transfer the dried **PC Column** to a new 1.5 ml microcentrifuge tube.
- Add **50-200 µl of Pre-Heated EL Buffer** or TE into the center of the column matrix.
- Let stand at 75ºC for 3 minutes.
- Centrifuge for 2 minutes at 14,000 x g to elute the purified DNA.



Troubleshooting

Problem	Cause	Solution
Low yield of DNA	Incompletely lysed sample	Use the required range or amount of starting materials to prepare the lysates.
		Increase the digestion time.
		Make sure that the tissue is completely immersed in the PL Buffer.
	Ethanol not added	Add absolute ethanol (see the bottle label for volume) to the W2 Buffer prior to use.
	Incorrect elution conditions	Perform incubation at 75°C for 3 minutes with Buffer EL before centrifugation. To recover more DNA, repeat the elution step.
	Poor quality of starting material	Be sure to use fresh sample and process immediately after collection or freeze the sample at -80°C or in liquid nitrogen. The yield and quality of DNA isolated depends on the type and age of the starting material.
DNA degradation	Sample not fresh	Avoid repeated freeze / thaw cycles of the sample. Use a new sample for DNA isolation. Perform the extraction using fresh materials whenever possible.
	DNase contaminantion	Maintain a sterile environment while working (e.g. wear gloves and use DNase-free reagents). Use fresh TAE or TBE electrophoresis buffer.
Inhibition of downstream enzymatic reactions	Purified DNA contains residual ethanol	If residual solution is seen in the purification column after washing the column with Buffer W2, empty the collection tube and re-spin the column for an additional 1 min. at the maximum speed (≥12000 x g).
	Purified DNA contains residual salt	Use the Wash Buffers in their correct order. Always wash the purification column with Buffer W1 first and then proceed to the wash step with Buffer W2.