Dual Genomic DNA Isolation Kit(Plant)

Cat No. PDC05-0100
Size: 100 Reactions

Sample: 100 mg of fresh plant tissue or 50 mg of dry plant tissue
Format: Reagent and spin column
Operation time: within 1 hour
Elution volume: 50~200 ul

Description

The **Dual Genomic DNA Isolation Kit (Plant)** combines reagent system and spin column system. The kit is designed specifically for isolating genomic DNA from plant samples. This unique reagent system ensures the total DNA with high yield and good quality from samples. The spin column system is designed to purify or concentrate DNA products which have been previously isolated with the Reagents. The entire procedure can be completed in 1 hour without phenol extraction. Purified DNA is suitable for use in PCR or other enzymatic reactions.

Kit Contents

Contents	PDC05-0100	PDDC05-0100S
Buffer PG	100 ml	4 ml
Buffer BD	100 ml	4 ml
Buffer W1	45 ml	2 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)	300 µl x2 (1.5 ml x2)
Buffer E	10 ml	1 ml
Columns DGP	100 pcs	4 pcs
Collection Tubes	100 pcs	4 pcs

Feature

- Delivering high-quality genomic DNA with the fast procedure
- Ready-to-use genomic DNA for high performance in any downstream application
- Highly purified and high yield genomic DNA can be extracted from various plant samples.
- Optimized plant lysis buffer for the efficient lysis.

Application

- Gene cloning, PCR
- Quantitative real time PCR
- Southern blotting
- SNP genotyping

Required Materials

- RNase A (50 mg/ml)
- Chloroform
- Mortar and pestle
- Microcentrifuge tubes
- Isopropanol
- Absolute ethanol
- Liquid nitrogen

Quality Control

The quality of the Dual Genomic DNA Isolation Kit is tested on a lot-to-lot basis to ensure consistent product quality.

Dual Genomic DNA Isolation Kit (Plant) Protocol

Step 1 Sample Preparation

1. Cut off 100 mg of fresh plant tissue or 50 mg of dry plant tissue.
2. Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.

Step 2 Lysis

1. Add 1 ml of Buffer PG 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.
2. Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.
3. Incubate at 75°C for 30 minutes.(invert the tube every 10 minutes)
4. Centrifuge at 14-16,000 x g for 5 minutes.
5. Transfer carefully the clear supernatant to a new 1.5 ml microcentrifuge tube.

Step 3 Phase Separation

1. Add 600 µl of chloroform to the supernatant from Step 2.
2. Shake vigorously and then centrifuge at 14-16,000 x g for 10 minutes.
3. Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube.
4. Repeat the Phase Separation Step until the interphase becomes clear then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube

∗The number of repetitions is dependent on sample type; e.g. dense tissue samples may require a higher number of repeats.

Step 4 DNA Precipitation

1. Add 800 µl of isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step3.
2. Mix the sample by inverting gently and let stand for 5 minutes at room temperature (DNA precipitation can be increased with extended standing time).
3. Centrifuge at 14-16,000 x g for 15 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of 70% ethanol.
5. Centrifuge at 14-16,000 x g for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 200 µl of TE Buffer or ddH_2O.
7. Incubate for 10 minutes at 75°C to dissolve the pellet.
8. If more pure DNA is required, perform this optional **DNA Pure Protocol.**

DNA Pure Protocol

✻ Add 60ml of the absolute ethanol to the Buffer W2 prior to initial use.
✻ Pre-heat the Buffer E to 75°C prior to use.

Step 1 Sample Prep.

1. Add 1 ml of Buffer BD to the sample which have been previously isolated using reagents and shake vigorously.

Step 2 DNA Binding

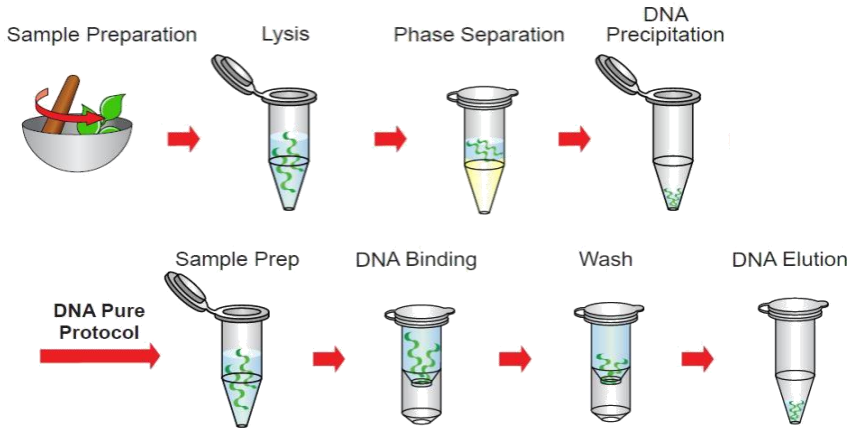
1. Place a Column DGP in a 2 ml Collection Tube.
2. Transfer the sample mixture from the previous step into the Column DGP.
3. Centrifuge at 14-16,000 x g for 30 seconds.
4. Discard the flow-through and place the Column DGP back in the same Collection Tube.
5. Centrifuge at 14-16,000 x g for 30 seconds.
6. Discard the flow-through and place the Column DGP back in the same Collection Tube.

Step 3 Wash

1. Add 400 µl of Buffer W1 into the Column DGP.
2. Centrifuge at 14-16,000 x g for 30 seconds.
3. Discard the flow-through and place the Column DGP back in the same Collection Tube.
4. Add 600 µl of Buffer W2 (ethanol added) into the Column DGP.
5. Centrifuge at 14-16,000 x g for 30 seconds.
6. Discard the flow-through and place the Column DGP back in the same Collection Tube.
7. Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

Step 4 DNA Elution

1. Transfer the dried Column DGP to a clean 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of Pre-Heated Buffer E or TE Buffer into the center of the column matrix.
3. Let stand at 75ºC for 5 minutes.
4. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when purifying the genomic DNA with the kit.

Problem	Cause	Solution
Low Yield of DNA	Incomplete 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 PG Buffer.
	Ethanol not added	Add the absolute ethanol to the Buffer W2 prior to the initial use.
		Make sure that the ethanol was added to the lysate before applying the sample to the Column DGP.
	Buffer E pH is too low	Check the pH.
	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 starting material quality	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 degrade	Buffer E not pre-heated at 75°C	Pre-heat the Buffer E to 75°C prior to use.
	Sample not fresh	Avoid repeated freeze / thaw cycles of the sample.
		Use a new sample for the DNA isolation. Perform the extraction of the fresh material when possible.
	DNase contaminant	Use the fresh TAE or TBE electrophoresis buffer.
Inhibition of downstream enzymatic reactions	Purified DNA containing residual ethanol	If the residual solution is seen in the purification column after washing the column with the 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 correct order for the Wash Buffers. Always wash the purification column with the Buffer W1 first, and then proceed to the wash with the Buffer W2.

Caution

- Check buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.