PG Reagent (Plant Genomic DNA Isolation Reagent)

Cat. No.: SN002-0100 Size: 100 Reactions
Cat. No.: SN002-0004 Size: 4 Reactions
Sample: Up to 100 mg of fresh tissue or 50 mg of dry plant tissue
Format: Reagent form
Operation time: 120 minutes
Elution volume: 50-200 µl

Description

The PG Reagent provides an easy 3-step method to isolate a high yield of total DNA (including genomic, mitochondrial, and chloroplast DNA) from the plant tissue and cells. This unique reagent is able to lyse the most common plant samples as well as samples with high polysaccharides. If a large sample is required, the reagent volume can be scaled up proportionally, making this reagent not only user-friendly but also highly versatile. The DNA phenol extraction is not required, and the entire procedure can be completed in 90 minutes. The extracted total DNA is ready for use in PCR, Real-time PCR, Southern Blotting, Mapping and RFLP.

Features

- Consistent DNA yields from a small amount of the starting material.
- Time flexibility.
- Ease of DNA extraction technique or method.
- Expense reduction.

Applications

- Quantity of DNA needed.
- Molecular weight and size of the DNA.
- Purity of DNA required.
- Downstream DNA applications.

Kit Content

Contents	SN002-0100	SN002-0004
PG Reagent	100 ml X 1 bottle	2 ml X 2 vials

Quality Control

The quality of the PG Reagent is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Homogenizer (mortar and pestle)
- Microcentrifuge tubes
- 70% ethanol
- Isopropanol
- RNase A (50 mg/ml)
- Chloroform

Buffer Preparation

TE Buffer (Tris-EDTA, pH8.0): 10 mM Tris-HCl, pH 8.0 with 0.1mM EDTA

Plant Genomic DNA Isolation Protocol

The standard protocol uses the PG Reagent for lysis of plant samples. For most common plant species, the reagent system ensures the isolated plant genomic DNA with a high yield and good quality.

Sample Preparation

1. Cut off the animal tissue (up to 30 mg) and transfer it to a 1.5 ml microcentrifuge tube.
2. Add 200 µl of the Grind Buffer to the tube and homogenize the sample tissue by grinding.
3. Cut off the fresh plant tissue (up to 100 mg) or the dry plant tissue (up to 50 mg).
4. Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.

Step 1 Lysis

1. Add 1 ml of the PG Reagent and 0.5 µl of the 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 65°C for 30-50 minutes.
4. Centrifuge at 14-16,000 x g for 10 minutes.
5. The supernatant would be transferred to a new 1.5 ml microcentrifuge tube.

Step 2 Phase Separation

Standard Samples

1. Add 600 µl of the chloroform to the supernatant from Step 1.
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, and then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.

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

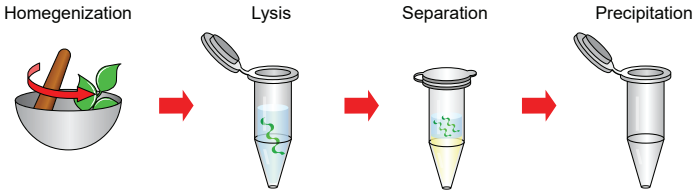
High Polysaccharide Samples

1. Add a 1/10 volume of the PG Reagent and 600 µl of the chloroform to the supernatant from Step 1.
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 and then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.

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

Step 3 DNA Precipitation

1. Add 800 µl of the isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step2.
2. Mix the sample by inverting gently and letting it stand for 5 minutes at the room temperature (DNA precipitation can be increased with the extended standing time).
3. Centrifuge at 14-16,000 x g for 20 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 50-200 µl of the TE buffer (no provided) or ddH_2O.
7. Incubate for 10 minutes at 60°C to dissolve the pellet.



Troubleshooting

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

Problem	Cause	Solution
Difficult to dissolve	Incomplete removal of ethanol	Remove ethanol in the hood briefly.
RNA containment	Incomplete removal of RNA	RNase A treatment.
Low yields of gDNA	Incomplete lysis and homogenization	Complete lysis and homogenization. Use the appropriate method for the lysate preparation based on the amount of the starting materials. Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the PG Reagent to achieve the optimal lysis.
	Presence of ethanol	Remove ethanol in the hood briefly.
	Incorrect elution conditions	Add TE Buffer or RNase-free H_2O (50-200 µl) and incubate for 10 min at 60°C.
	Incorrect separation phase	Repeat the Phase Separation Phase until the interphase becomes clear, and then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Remove ethanol in the hood briefly

Related Ordering Information

Cat. No.	Description	Size
SM101-0500	*Taq* DNA polymerase	500 U
SM200-0100	PCR SUPERMIX	100 Reactions
SM201-0100	Hot Start SUPERMIX	100 Reactions
SM255-0100	Ultrapure Proteinase K	100 mg
SL001-1000	Novel Juice (Supplied in 6X Loading Buffer)	1 ml
SD003-R500	100 bp DNA Ladder H3 RTU	500 µl
SD010-R500	1 Kb DNA Ladder RTU	500 µl
SD013-R500	XLarge DNA Ladder RTU	500 µl
ST040-4000	100 mM dNTP Set	4 x1 ml
ST046-1000	100 mM dNTP Set	4 x 250 µl
ST025-1000	2.5 mM dNTP Mix	1 ml
ST100-1000	25 mM dNTP Mix	1 ml

Caution

- Check buffers before use for salt precipitation.
- Re-dissolve any precipitate by warming up to 37°C.
- During the operation, always wear a lab coat, disposable gloves, and protective equipment.
- All products are for research use only.