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Product Information

GenElute™ Plant Genomic DNA Miniprep Kit

Product Codes **G2N10**, **G2N70**, and **G2N350**
Technical Bulletin Code MB-580

TECHNICAL BULLETIN

Product Description

Sigma's GenElute™ Plant Genomic DNA Miniprep Kit provides a simple and convenient way to isolate pure DNA from a variety of plant species. The GenElute kit combines the advantages of a silica-based system with a microspin format and eliminates the need for expensive resins, RNase treatment, and hazardous organic compounds such as phenol and chloroform.

Several micrograms of DNA can be obtained from up to 100 mg of fresh tissue or 10 mg of freeze-dried material in less than an hour. The purified DNA is greater than 20 kb in length and can be used in sensitive downstream applications such as restriction endonuclease digests and PCR[†] amplification.

Reagents Provided	Product Code	G2N10 10 Preps	G2N70 70 Preps	G2N350 350 Preps
Lysis Solution Part A	L 7910	4 ml	30 ml	140 ml
Lysis Solution Part B	L 8035	1 ml	4 ml	20 ml
Precipitation Solution	P 9727	1.5 ml	11 ml	50 ml
Binding Solution	B 2177	8 ml	60 ml	280 ml
Column Preparation Solution	C 2112	7 ml	60 ml	225 ml
Wash Solution Concentrate	W 3011	4 ml	30 ml	140 ml
Elution Solution (10 mM Tris, 1 mM EDTA, pH approx. 8.0)	T 7688	3 ml	20 ml	100 ml
GenElute™ Filtration Columns in Tubes	C 9346	10 each	70 each	5 x 70 each
GenElute™ Nucleic Acid Binding Columns in Tubes	C 9471	10 each	70 each	5 x 70 each
Collection Tubes, 2.0 ml capacity	T 7813	3 x 10 each	3 x 70 each	15 x 70 each

Storage

Store the kit at room temperature. If any kit reagent forms a precipitate upon storage, warm at 65 °C until the precipitate dissolves.

Reagents and Equipment Required But Not Provided

- Small mortar and pestle
- Liquid nitrogen
- Microcentrifuge tubes
- Microcentrifuge
- RNase A Solution, Product Code R 4642
- Ethanol, 95% or 100%, Product Codes E 7023, E 7148, or 45,983-6
- Molecular biology reagent water, Product Code W 4502
- 65 °C water bath

Precautions and Disclaimer

The GenElute Plant Genomic DNA Miniprep Kit is for laboratory use only. Not for drug, household or other uses. The Lysis Solution [Part A] and the Binding Solution contain guanidine thiocyanate, which is harmful. The Column Preparation Solution is an irritant. Avoid contact with skin. Wear gloves, safety glasses, and suitable protective clothing when handling these solutions or any reagent provided with the kit. Please consult the Materials Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Preparation Instructions

1. Thoroughly mix reagents. Examine reagents for precipitation. If any reagent forms a precipitate, warm at 55-65 °C until the precipitate dissolves and allow to cool to room temperature before use.
2. Preheat the water bath to 65 °C.
3. Wash Solution: Dilute the Wash Solution Concentrate with 9.5 ml (10 prep package), 72 ml (70 prep package), or 330 ml (350 prep package) of 95-100% ethanol. After each use, tightly cap the diluted wash solution to prevent the evaporation of ethanol.
4. Preheat the Elution Solution to 65 °C.

Procedure

1. Disrupt cells. Grind plant tissue into a fine powder in liquid nitrogen using a mortar and pestle. Transfer up to 100 mg of the powder to a Microcentrifuge tube. Keep the sample on ice for immediate use or freeze as -70 °C.
2. Lyse cells. Add 350 µl of Lysis Solution [Part A] and 50 µl of Lysis Solution [Part B] to the tube; thoroughly mix by vortexing and inverting. A white precipitate will form upon the addition of Lysis Solution (Part B). Incubate the mixture at 65 °C for 10 minutes with occasional inversion to dissolve the precipitate.

Optional digest with RNase.

This kit is designed to selectively isolate large DNA. If preparations are found to be contaminated with RNA, RNase A (not supplied) can be used to digest the RNA. Add 50 units of RNase A to the lysis mixture just prior to incubation at 65 °C.

3. Precipitate debris. Add 130 µl of Precipitation Solution to the mixture; mix completely by inversion and place the sample on ice for 5 minutes. Ice at maximum speed (12,000-16,000 x g) for 5 minutes to pellet the cellular debris, proteins, and polysaccharides.
4. Filter debris. Carefully pipette the supernatant from step 3 onto a GenElute filtration column (blue insert with a 2 ml collection tube). Centrifuge at maximum speed for 1 minute. This removes any cellular debris not removed in step 3. Discard the filtration column, but retain the collection tube.
5. Prepare for binding. Add 700 µl of Binding Solution directly to the flow-through liquid from step 4. Mix thoroughly by inversion.
6. Prepare binding column. Insert a GenElute Miniprep Binding Column (with a red o-ring) into a provided microcentrifuge tube, if not already assembled. Add 500 µl of the Column Preparation Solution to each miniprep column and centrifuge at 12,000 x g for 30 seconds to 1 minute. Discard the flow-through liquid

Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.

7. Load lysate. Carefully pipette 700 µl of the mixture from step 5 onto the column prepared in step 6 and centrifuge at maximum speed for 1 minute. Discard the flow-through liquid; retain the collection tube. Return the column to the collection tube. Apply the remaining lysate from step 5 onto the column. Repeat the centrifugation as above and discard the flow-through liquid and collection tube.
8. First Column Wash. Prior to first time use, be sure to add ethanol to the Wash Solution Concentrate. Place the binding column into a fresh 2 ml collection tube and apply 500 µl of the diluted Wash Solution to the column. Centrifuge at maximum speed for 1 minute. Discard the flow-through liquid, but retain the collection tube.
9. Second Column Wash. Apply another 500 µl of diluted Wash Solution to the column and centrifuge at maximum speed for **3 minutes** to dry the column. Do not allow the flow-through liquid to contact the column; wipe off any fluid that adheres to the outside of the column.

Elute DNA:

Transfer the binding column to a fresh 2 ml collection tube. Apply 100 μ l of pre-warmed (65 °C) Elution Solution to the column and centrifuge at maximum speed for 1 minute. Repeat the elution. Do not allow the flow-through liquid to contact the column. Eluates may be collected in the same collection tube. Alternatively, a second collection tube may be used for the second elution to prevent dilution of the first eluate.

The eluate contains pure genomic DNA. For short-term storage of DNA, 2-8 °C is recommended. For long-term storage of DNA, -20 °C is recommended. Avoid freezing and thawing, which causes breaks in the DNA strand. Elution Solution will help stabilize the DNA at these temperatures.

DNA Precipitation (Optional)

The GenElute Plant Genomic DNA Kit is designed so that the DNA remains in solution, thus avoiding resuspension problems. However, if it is necessary to concentrate the DNA, ethanol precipitation in the presence of sodium acetate is recommended.¹

Alternative Disruption Procedures

The extraction of nucleic acid from plant tissue is complicated by the tough cell wall that surrounds most plant cells as well as the fibrous nature of many species. Several methods exist for the disruption of plant material. One of the most effective and commonly used methods is to grind the tissue in liquid nitrogen with a mortar and pestle. The GenElute Plant Genomic DNA Miniprep Kit was developed based on

this efficient method of disruption. However, other disruption techniques can be substituted for step 1 of the Procedure.

Good yields of high molecular weight DNA can also be obtained from freeze-dried tissue. Dried tissue should be ground into a fine powder with a mortar and pestle; up to 20 mg of this powder can be used in a single DNA preparation. Liquid nitrogen is not necessary during the grinding of freeze-dried tissue. After grinding the tissue into a powder, follow the Procedure beginning with step 2.

Results

Determine the concentration and purity of the plant DNA by spectrophotometric analysis and agarose gel electrophoresis. The ratio of absorbance at 260 nm to 280 nm (A_{260}/A_{280}) should be 1.7 to 1.9. The size and quality of the DNA can be determined by agarose gel electrophoresis or pulsed field electrophoresis.

References

1. Sambrook, J., *et al. Molecular Cloning: A Laboratory Manual*, 2nd ed., pp. 6.2-6.19, (Cold Spring Harbor Laboratory Press, Plainview, NY, E10-E14. 1989)
2. Birren, B. and Lai, E., *Pulsed Field Gel Electrophoresis: A Practical Guide* (Academic Press, San Diego, CA, 1993)

Table 2. Typical yields of DNA from various plant species per 100 mg of tissue:

Plant	DNA Yield	Plant	DNA Yield
Corn	7.5 μ g	Tobacco	5.2 μ g
<i>Dianthus</i> tissue culture	3.3 μ g	Tomato	6.2 μ g
Pepper	3.1 μ g	Tomato (20 mg of freeze-dried leaf tissue)	5.7 μ g
Rice	5.9 μ g		
Soybean	5.7 μ g	Wheat	11.5 μ g

Troubleshooting Guide

Problem	Cause	Solution
Binding column is clogged.	Sample is too large.	For future preparations, use less plant tissue. To salvage the current preparation, increase the <i>g</i> -force and/or spin longer until lysate passes through the binding column. The DNA yield may be reduced.
	Tissue is insufficiently disrupted.	Thoroughly disrupt the starting material according to step 1 of the protocol. If using alternative methods, make sure that you are effectively disrupting the tissue.
DNA yield is low.	Sample may be old or degraded.	Yields will vary between different types of plant tissues and plant species. If possible, use the youngest leaves or tissues. If samples are being stored for future use, flash-freeze in liquid nitrogen and store at $-70\text{ }^{\circ}\text{C}$.
	Tissue is insufficiently disrupted.	See above.
	The eluate contains residual ethanol from wash.	Ethanol from the final wash must be eliminated before eluting the DNA. A longer or additional spin, as recommended in step 9, is required to dry the membrane. If the flow-through liquid containing ethanol contacts the binding column, repeat the centrifugation step before eluting the DNA.
	Wash Solution Concentrate was not diluted before use.	Confirm that the Wash Solution Concentrate was properly diluted with ethanol before use.
	DNA elution is incomplete.	Confirm that the DNA was eluted in 100 μl of Elution Solution. A second and third elution using 100 μl of Elution Solution may be performed.
	Water was used for elution instead of Elution Solution.	Elution Solution is recommended for optimal yields and storage of the purified DNA. If water is used to elute the DNA, confirm that the pH is at least 7.0, to avoid acidic conditions which may subject the DNA to acid hydrolysis when stored for long periods of time.
Purity of the DNA is lower than expected: A_{260}/A_{280} ratio is too low.	Purification is incomplete.	Reduce the initial volume of the sample.
	Background reading is high due to silica fines.	Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
Purity of the DNA is lower than expected: A_{260}/A_{280} ratio is too high.	Genomic DNA is contaminated with RNA.	Include an RNase A treatment in future isolations or treat the final product with RNase A Solution and repurify.

Troubleshooting guide (continued)

Problem	Cause	Solution
DNA is sheared	DNA sample was excessively manipulated	All pipetting steps should be accomplished as gently as possible. Wide-bore pipette tips are recommended to help eliminate potential shearing. Do not vortex.
	Sample is old, degraded, or has undergone repeated freeze/thaw cycles	Old starting material may yield degraded DNA in the eluate. Fresh preparations should be used immediately or be frozen in liquid nitrogen and stored at -70°C until needed.
Downstream applications are inhibited	Ethanol is carried over into the final genomic DNA preparation	After the final wash of the binding column (step 9) do not allow the flow-through liquid to contact the column. Re-spin the column, if necessary, after emptying the collection tube, for an additional 1 minute at maximum speed (12,000-16,000 $\times g$).
	Salt is carried over into the final genomic DNA preparation	Make sure that binding column is transferred to a new collection tube before adding the Wash Solution in step 8. Wash twice with 500 μl of Wash Solution.

Related Products	Product Code	Related Books	Product Code
Agarose	A 9539	<i>Methods in Plant Molecular Biology: A Laboratory Course Manual</i> , Maliga, P. et al., Cold Springs Harbor Laboratory Press, CSH, NY, 1995.	Z37,375-3
Ethidium bromide, 10 mg/ml	E 1510		
Lambda DNA <i>EcoR</i> I <i>Hind</i> III marker	D 9281		
Microcentrifuge tubes, 1.5 ml	T 9661	<i>Plant Molecular Biology: A Laboratory Manual</i> . U.S. Clark, Ed. Springer-Verlag, NY, NY, 1997.	Z37,550-0
PCR Core Kit with <i>Taq</i> polymerase	CORE-T		
Pipet tips, 200 μl , wide orifice	P 1678		
RNase A Solution	R 4642	<i>Plant Molecular Biology: Labfax</i> . R.R.R. Croy, Ed., Bios Scientific Publishers, Oxford, England, 1994.	Z35,746-4
Sodium acetate, 3M	S 7899		
<i>Taq</i> DNA polymerase	D 1806, D 4545		
TBE buffer, 5x concentrate	T 6400		

†The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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