User Guide

Catalog Nos.
G2N10
G2N70
G2N350

GenElute™ Plant Genomic DNA Miniprep Kit

sigma.com

Ordering Information

Catalog Number	Product Description	Pkg Size
G2N10	GenElute Plant Genomic DNA Miniprep Kit	10 preps
G2N70	GenElute Plant Genomic DNA Miniprep Kit	70 preps
G2N350	GenElute Plant Genomic DNA Miniprep Kit	350 preps

To reorder product call 1-800-325-3010, visit our Web site at sigma-aldrich.com, or contact your local sales representative.

GenElute Plant Genomic DNA Miniprep Kit

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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	Cat. No.	G2N10 10 Preps	G2N70 70 Preps	G2N350 350 Preps
Lysis Solution Part A	**L7910**	4 mL	30 mL	140 mL
Lysis Solution Part B	**L8035**	1 mL	4 mL	20 mL
Precipitation Solution	**P9727**	1.5 mL	11 mL	50 mL
Binding Solution	**B2177**	8 mL	60 mL	280 mL
Column Preparation Solution	**C2112**	7 mL	60 mL	225 mL
Wash Solution Concentrate	**W3011**	4 mL	30 mL	140 mL
Elution Solution (10 mM Tris, 1 mM EDTA, pH approx. 8.0)	**T7688**	3 mL	20 mL	100 mL
GenElute Filtration Columns	**CP9346***	10 each	70 each	5 × 70 each
GenElute Nucleic Acid Binding Columns	**CP9471***	10 each	70 each	5 × 70 each
Collection Tubes, 2.0 mL capacity	**T5449**	4 × 10 each	4 × 70 each	20 × 70 each

*MilliporeSigma continually seeks ways to improve our products. Please note that the product codes for the GenElute Nucleic Acid Binding Columns and GenElute Filtration Columns have changed from C9471 to CP9471 and C9346 to CP9346, respectively. These changes have been made to streamline and make more consistent, all the GenElute products across the line. The performance and functionality of the C9471 & CP9471 binding columns and the C9346 & CP9346 filtration columns are equivalent.

Equipment and Reagents Required But Not Provided

- Small mortar and pestle
- Liquid nitrogen
- Microcentrifuge tubes
- Microcentrifuge (2 mL tube, rotor equipped)**
- RNase A Solution, Catalog No. **R4642**
- Ethanol, 95%, or 100%, Catalog Nos. **E7023**, **E7148**, or **459836**
- Molecular biology reagent water, Catalog No. **W4502**
- 65 °C water bath

****Note:** To ensure proper fit of all tubes, a 24-place rotor is recommended. If you are using a 36-place rotor, we recommend using every other place for proper tube fit.

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.

Storage and Stability

Store the kit at room temperature. If any kit reagent forms a precipitate upon storage, warm at 55–65 °C until the precipitate dissolves.

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 (**W3011**) 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] (**L7910**) and 50 µL of Lysis Solution [Part B] (**L8035**) 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 (**P9727**) to the mixture; mix completely by inversion and place the sample on ice for 5 minutes. Centrifuge the sample at maximum speed (12,000–16,000 × *g*) for 5 minutes to pellet the cellular debris, proteins, and polysaccharides.

4. Filter Debris

Assemble a filtration column (**CP9346**) with a 2 mL collection tube (**T5449**). Carefully pipette the supernatant from step 3 onto the filtration column. 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 (**B2177**) directly to the flow-through liquid from step 4. Mix thoroughly by inversion.

6. Prepare Binding Column

Assemble a binding column (**CP9471**) with a 2 mL collection tube (**T5449**). Add 500 µL of the Column Preparation Solution (**C2112**) to the binding column and centrifuge at 12,000 × *g* for 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 (**W3011**). 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 (12,000–16,000 × *g*) for **3 minutes** to dry the column. The column must be free of ethanol before eluting the DNA. Centrifuge the column for an additional 1 minute at maximum speed if residual ethanol is seen. You may empty and re-use the collection tube if you need this additional centrifugation step. Finally, discard the collection tube containing the flow-through liquid and place the binding column in a new 2 mL collection tube.

10. Elute DNA

Pipette 100 µL of pre-warmed (65 °C) Elution Solution (**T7688**) directly onto the center of the column and centrifuge at maximum speed for 1 minute. Repeat the elution. Eluates may be collected in the same collection tube. Alternatively, a second collection tube (not provided) 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 Blood Genomic DNA Kit is designed so that the DNA always remains in solution, which avoids resuspension issues. 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.

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

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

References

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

Troubleshooting Guide

Binding column is clogged.	**Cause** — Sample is too large. **Solution** — For future preparations, use less plant tissue. To salvage the current preparation, increase the *g*-force and/or spin longer until lysate passes through the binding column. The DNA yield may be reduced.
	Cause — Tissue is insufficiently disrupted. **Solution** — 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.	**Cause** — Sample may be old or degraded. **Solution** — 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 °C.
	Cause — Tissue is insufficiently disrupted. **Solution** — 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 °C.
	Cause — The eluate contains residual ethanol from wash. **Solution** — 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.
	Cause — Wash Solution Concentrate was not diluted before use. **Solution** — Confirm that the Wash Solution Concentrate was properly diluted with ethanol before use.
	Cause — DNA elution is incomplete. **Solution** — 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.
	Cause — Water was used for elution instead of Elution Solution. **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.	**Cause** — Purification is incomplete. **Solution** — Reduce the initial volume of the sample.
	Cause — Background reading is high due to silica fines. **Solution** — 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.	**Cause** — Genomic DNA is contaminated with RNA. **Solution** — Include an RNase A treatment in future isolations or treat the final product with RNase A Solution and repurify.
DNA is sheared.	**Cause** — DNA sample was excessively manipulated. **Solution** — All pipetting steps should be accomplished as gently as possible. Wide-bore pipette tips are recommended to help eliminate potential shearing. Do not vortex.
	Cause — Sample is old, degraded, or has undergone repeated freeze/thaw cycles. **Solution** — 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.	**Cause** — Ethanol is carried over into the final genomic DNA preparation **Solution** — 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 × *g*).
	Cause — Salt is carried over into the final genomic DNA preparation. **Solution** — 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	Catalog No.	Related Products	Catalog No.
Agarose	**A9539**	Pipette tips, 200 µL, wide orifice	**P1678**
Ethidium bromide, 10 mg/mL	**E1510**	RNase A Solution	**R4642**
Lambda DNA *Eco*R I *Hin*d III marker	**D9281**	Sodium acetate, 3 M	**S7899**
Microcentrifuge tubes, 1.5 mL	**T9661**	*Taq* DNA polymerase	**D1806, D4545**
PCR Core Kit with *Taq* polymerase	**CORET**	TBE buffer, 5× concentrate	**T6400**

Notes

Experienced User Protocol

1 Prepare Plant Tissue

- ❒ Grind in liquid nitrogen.

2 Release DNA

- ❒ Lyse, precipitate, and spin.

3 Filter Lysate

- ❒ Spin 1 minute.

4 Prepare Column

- ❒ Add solution and spin.

5 Bind DNA

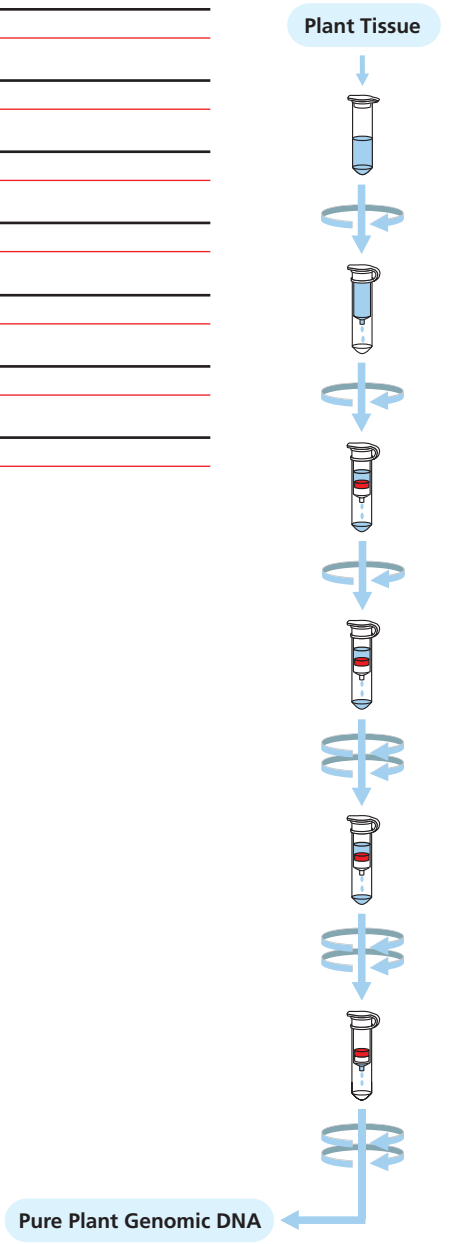
- ❒ Bind and spin twice.

6 Wash Column

- ❒ Wash and spin twice.

7 Elute DNA

- ❒ Elute and spin twice.



World Headquarters
3050 Spruce St., St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052
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