Instruction Manual
Research Use Only

SPINeasy DNA Kit for Plant

Spin Column Purification for Easy Isolation of genomic DNA from Plant Tissue

Size: 50 & 5 preps
Storage: 15-25 °C
Cat. No.: 116535050 (50 PREPS)
116535005 (5 PREPS)
Content Version: November 2023

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1. Introduction to SPINeasy DNA Kit for Plant

SPINeasy DNA Kit for Plant is a silica-membrane spin-column kit that enables quick, convenient, and cost-effective isolation of genomic DNA (gDNA) from various plant tissues, such as leaf, stem and seed. High quality gDNA can be extracted from polysaccharide and polyphenolic-rich plants. The use of our specially formulated Lysis Buffer PD and Lysing Matrix A in combination with FastPrep® Instruments from MP Biomedicals enables highly efficient lysis of various types of samples within seconds. Provided in the kit, Column PD and kit buffers are designed to deliver gDNA extracts of high yield and purity, compatible with downstream molecular biology applications such as PCR, restriction digestion and sequencing. Visit www.mpbio.com to explore additional products to support your research.

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Mini spin column
Sample	Plant
Sample amount	50 – 150 mg
Elution volume	100 µL

2. Kit Components and User Supplied Materials

2.1 SPINeasy DNA Kit for Plant Component

Components	50 PREPS (Cat.No.: 116535050) Package	Cat. No.
Equilibration Buffer	12 mL	116547059
Lysing Matrix A	50 ea	116910050
Lysis Buffer PD	50 mL	116535051
PPS Solution	12 mL	116535052
Binding Buffer PD	15 mL	116535053
Wash Buffer PD	2 x 12 mL	116535054
Elution Buffer PD	10 mL	116535056
RNase A	0.8 mL	116535057
Column PD with collection tubes	50 ea	116535058
Quick-Start Protocol	1 ea	-
Instruction Manual	Available www.mpbio.com	
MSDS & CoA	Available www.mpbio.com	

2.2 User Supplied Materials

- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500)
- Vortex mixer with adapter
- Microcentrifuge capable of at least 14,000 *g*
- Isopropanol (35 mL)
- Absolute ethanol (56 mL)
- 1.5 mL microcentrifuge tubes
- 2.0 mL microcentrifuge tubes
- Heat block (optional)
- Single-channel pipettors (1 µL–1000 µL)
- Nuclease-free, aerosol-preventive tips
- Rack for microcentrifuge tube
- Biohazard disposal containers
- Personal Protective Equipment

3. Storage and Kit Stability

All the components and reagents of the SPINeasy DNA Kit for Plant (With Lysing Matrix) can be stored at room temperature (15-25˚C) until the expiration date printed on the kit label. For extended storage or storage in dry condition (humidity < 40%), store the columns at 2-8˚C to maintain performance.

4. Important Consideration Before Use

- ❑ Add 35 mL (3.5 mL for sample kit) of isopropanol to Binding Buffer PD and mark the bottle.
- ❑ Add 28 mL (5.6 mL for sample kit) of absolute ethanol to each bottle of Wash Buffer PD and mark the bottles.
- ❑ Prepare two 1.5 mL microcentrifuge tubes per prep: one for lysate preparation and another for elution of purified genomic DNA.
- ❑ Prepare a 2 mL microcentrifuge tube per prep for sample binding preparation.
- ❑ Lysis can be performed by vortexing the sample in a vial of Lysing Matrix A at the maximum speed if a FastPrep® Instrument is unavailable.
- ❑ Centrifugation speed stated in the manual will be a guideline; use the maximum speed available if 14,000 *g* is not feasible.

5. Safety Precautions

Binding Buffer PD contains a component that can be harmful if swallowed and may cause irritation when in contact with skin and eyes. To prevent accidental ingestion, do not eat, drink, or smoke when using this product. Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details.

6. Protocol

1. **Column PD Preparation**

   - Add **200 µL Equilibration Buffer** to the **Column PD** membranes to ensure its performance.

     Wait at least **1 min** and centrifuge for **30 sec @ 14,000 *g***. Discard flow through and reuse collection tube.

2. **Homogenize**

   - Weigh **50 mg – 150 mg** of sample and transfer into a vial of **Lysing Matrix A**.
   - Add **900 µL Lysis Buffer PD**. ***Optional:*** *Add 15 µL RNase A.*
   - Homogenize in a FastPrep® Instrument with a speed setting of **4.0 m/s** for **20 sec.**
   - While this setting is found to be suitable for most sample types tested, lysing speed and duration may be adjusted to achieve optimal performance for each sample type.
   - If a FastPrep® Instrument is unavailable, cut sample into small pieces, or grind samples in liquid nitrogen before transferring into Lysing Matrix A and vortex for **10 – 15 min @ the maximum speed**. It is recommended to perform vortexing with the use of an adapter to hold the vials, especially if multiple samples are to be processed simultaneously.
   - Centrifuge for **10 min @ 14,000 *g***.

     Note: *Centrifuge at the maximum speed for all steps if **14,000 g** is not feasible.*
   - Transfer **700 µL** lysate supernatant into a clean 1.5 mL microcentrifuge tube and add **200 µL PPS Solution**. Mix well by inverting the tube several times.
   - Centrifuge for **1 min @ 14,000 *g***.

3. **Bind**

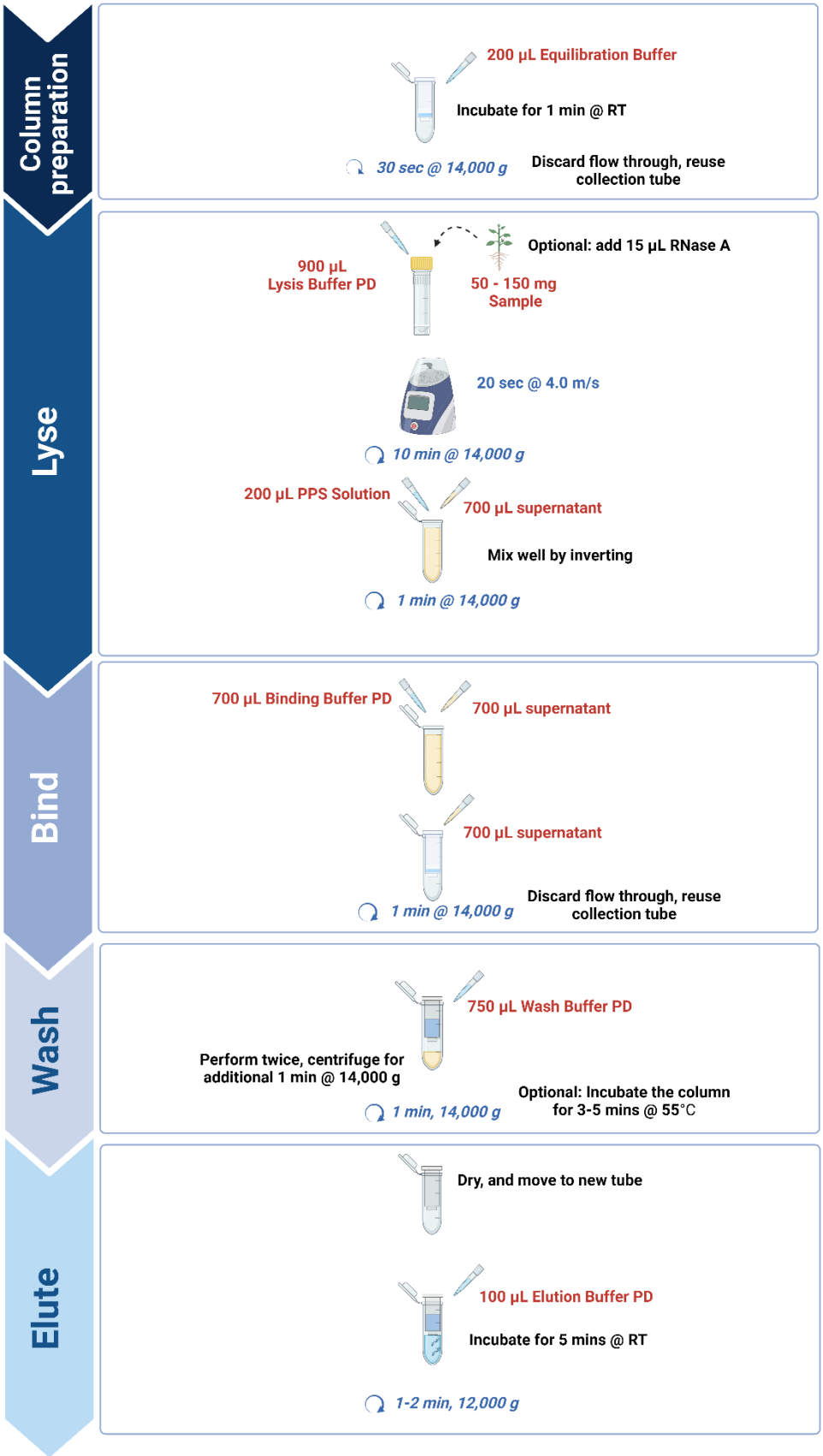
   - Transfer **700 µL** supernatant into a clean 2 mL microcentrifuge tube and add **700 µL Binding Buffer PD**. Mix by pipetting up and down or inverting the tube several times.
   - Transfer **700 µL** supernatant to a Column PD with collection tube.
   - Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
   - Repeat to load the remaining supernatant.

4. **Wash**
   - Add **750 µL Wash Buffer PD** to the column.
   - Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
   - Repeat the washing step once.
   - Centrifuge for **an additional 1 min @ 14,000 *g*** to dry column.

     Optional: *Incubate for **3 - 5 min @ 55 °C** to dry column completely.*

5. **Elution**
   - Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.
   - Add **100 µL Elution Buffer PD** to the center of the membrane. Incubate for **5 min @ room temperature**. For samples with low DNA content, reducing the elution volume to 50 µL may increase the concentration of the eluted DNA.
   - Centrifuge for **1 min @ 12,000 *g*** to elute DNA.
   - Eluted genomic DNA will be collected in the microcentrifuge tube.

7. Flow Chart


Column preparation
200 µL Equilibration Buffer
Incubate for 1 min @ RT
30 sec @ 14,000 g
Discard flow through, reuse collection tube
Lyse
Optional: add 15 µL RNase A
900 µL Lysis Buffer PD
50 - 150 mg Sample
20 sec @ 4.0 m/s
10 min @ 14,000 g
200 µL PPS Solution
700 µL supernatant
Mix well by inverting
1 min @ 14,000 g
Bind
700 µL Binding Buffer PD
700 µL supernatant
700 µL supernatant
1 min @ 14,000 g
Discard flow through, reuse collection tube
Wash
750 µL Wash Buffer PD
Perform twice, centrifuge for additional 1 min @ 14,000 g
1 min, 14,000 g
Optional: Incubate the column for 3-5 mins @ 55°C
Elute
Dry, and move to new tube
100 µL Elution Buffer PD
Incubate for 5 mins @ RT
1-2 min, 12,000 g

8. Data

SPINeasy DNA Kit for Plant has been rigorously tested for its performance. The following table displays gDNA yields obtained from various samples using the kit. Results demonstrate that gDNA extracted with this kit is suitable for PCR amplification.

Table 1: Quality and quantity of gDNA extracted from various plant tissues using SPINeasy DNA Kit for Plant.

Sample	Starting Amount	Extraction Results		
		Concentration (ng/µL)	$A_{260/280}$	$A_{260/230}$
Pothos leaf	100 mg	16.81±3.40	1.78±0.00	1.78±0.30
Tomato leaf	100 mg	37.50±8.30	1.81±0.02	2.08±0.06
Chili leaf	100 mg	42.23±0.77	1.81±0.00	2.19±0.07
Tobacco leaf	100 mg	28.41±3.09	1.80±0.00	2.06±0.01
Pine needle	100 mg	72.07±2.01	1.82±0.01	2.02±0.02
Corn leaf	50 mg	50.64±5.71	1.83±0.00	2.22±0.01
Corn kernel	1 grain	77.45±7.63	1.83±0.00	1.98±0.03

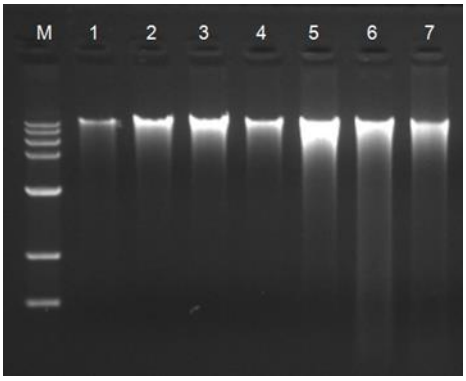


Figure 1: (left) gDNA extracted from various samples using SPINeasy DNA Kit for Plant, analyzed using agarose gel electrophoresis.
M: DNA marker; Lane 1: Pothos leaf; Lane 2: Tomato leaf; Lane 3: Chili leaf; Lane 4: Tobacco leaf; Lane 5: Pine needle; Lane 6: Corn leaf; Lane 7: Corn kernel.

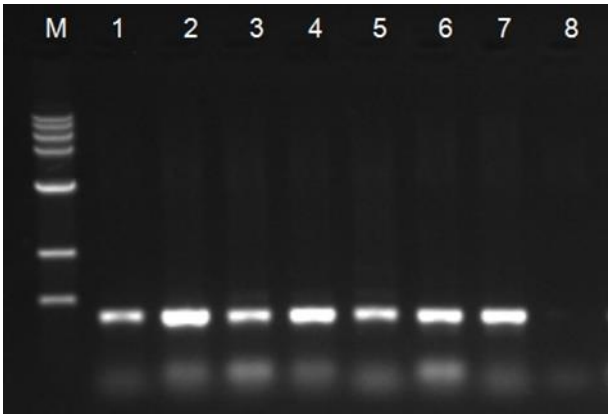


Figure 2: (right): PCR amplification of gDNA extracted from various samples using SPINeasy DNA Kit for Plant.
M: DNA marker; Lane 1: Pothos leaf; Lane 2: Tomato leaf; Lane 3: Chili leaf; Lane 4: Tobacco leaf; Lane 5: Pine needle; Lane 6: Corn leaf; Lane 7: Corn kernel; Lane 8: Negative control.

9. Troubleshooting

This guide may be useful in solving any problems that may arise. For further assistance, please contact our technical support team at **apac-techsupport@mpbio.com**

Problem	Possible Cause	Recommendation
Low DNA Yield	Inefficient extraction	Ensure the extraction was performed according to the kit manual's instructions.
	Tissue with low DNA content	(i) Increase amount of starting material; (ii) Process multiple samples using several Lysing Matrix tubes and then pool the samples; (iii) Elute in a smaller volume (50 µL).
	Insufficient lysis	While a FastPrep® speed setting of 4.0 m/s for 20 seconds is sufficient for most sample types, some samples may require harsher conditions for complete lysis. Homogenization speed and/or time can be increased for such samples. When lysis is performed using a vortex instead of a FastPrep®, samples can be pre-homogenized by grinding in liquid nitrogen before vortexing in a Lysing Matrix. Vortexing duration can also be extended as necessary.
	Poor elution	Ensure that Elution Buffer PD is added to the center of the column membrane.
	Ethanol carry-over	Incubate column at 55 °C for 3 – 5 mins to dry the membrane completely before elution.
Low A_{260}/A_{280} ratio	Inaccurate readings due to low DNA concentration	Ensure the extraction was performed according to the kit manual's instructions.
	Insufficient cell lysis	While a FastPrep® speed setting of 4.0 m/s for 20 seconds is sufficient for most sample types, some samples may require harsher conditions for complete lysis. Homogenization speed and/or time can be increased for such samples. When lysis is performed using a vortex instead of a FastPrep®, samples can be pre-homogenized by grinding in liquid nitrogen before vortexing in a Lysing

		Matrix. Vortexing duration can also be extended as necessary.
	Contaminants not removed efficiently	Ensure that washing step is performed twice with Wash Buffer PD.
High A_{260}/A_{280} ratio	Inaccurate readings due to low DNA concentration	Ensure the extraction was performed according to the kit manual's instructions.
	RNA contamination	Add RNase A in Step 2 of DNA extraction.
Low A_{260}/A_{230} ratios	Inaccurate readings due to low DNA concentration	Ensure the extraction was performed according to the kit manual's instructions.
	Contaminants not removed efficiently	Ensure that washing step is performed twice with Wash Buffer PD.
Sheared DNA	Sample is over-lysed	Reduce FastPrep® lysis speed and/or duration.
	Sample degradation	Sample quality is critical to the integrity of purified DNA. For best results, DNA should be extracted from fresh samples.
RNA Contamination	Missed addition of RNase A	Add RNase A in Step 2 of DNA extraction.

10. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices to diagnose, cure, mitigate, treat, or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery.

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