Plant gDNA Miniprep Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

The mdi Plant gDNA Miniprep Kit is a fast, economical and easy isolation method of high purity gDNA from plant tissues. The buffer system provided in the kit allows tissue lysis followed by selective binding of gDNA to the spin column.

Purified gDNA is eluted in low-salt buffer or water for variety of downstream applications. This technology does away with the cumbersome methodologies of phenol extraction (associated with slurries formation) as well as ethanol precipitation (associated with anion exchange based purification system) for desalting.

2. Applications

1. Automated Fluorescent Sequencing
2. Radioactive Sequencing
3. Restriction Digestion
4. Cloning
5. PCR

3. Storage Conditions

Optimum storage conditions at which all components of the kit can be preserved without alteration in it's quality and performance.

One Year	RT
Longer Storage	2-8°C

Important

In case of any precipitation observed in the buffers, re-dissolve by warming at 37°C for sometime and cool it down to room temperature.

4. Quality Assurance

The mdi Plant gDNA Miniprep kit is designed for various pre-determined specifications and user requirements such as yield, purity, ruggedness, shelf life and functional convenience.

These are produced through a well defined quality management system certified by Underwriters Laboratories, USA for ISO 9001: 2008 which ensures intra lot as well as lot to lot consistency.

5. Safety Information

The buffers and the reagents may contain irritants, so wear lab coat, disposable gloves and protective goggles while working with the Plant gDNA Miniprep Kit.

6. Lot Release Criteria

Each lot of Plant gDNA Miniprep Kit is tested against predetermined specifications to ensure consistent product quality.

7. Technical Support

At mdi, customers are our priority. We will share our experiences to assist you to overcome problems in general product usage as well as customize products for special applications. We will

* Stimulate problems, and suggest alternative methods to solve them.
* Make changes/ improvements in our existing products/protocols.
* Develop special new products and systems especially to satisfy your needs.

We welcome your feedback to improve our products.

8. Kit Contents

Contents	Quantity			Storage Temperature
Plant Mini Spin Columns	50	250	1000	RT
mdi Shredder Mini Spin Columns	50	250	1000	RT
Buffer PL 1	36ml	180ml	720ml	RT
Buffer PL 2	18ml	90ml	360ml	RT
Buffer PL 3	36ml	180ml	720ml	RT
Buffer PLW	60ml	300ml	1200ml	RT
Buffer PLE	24ml	120ml	480ml	RT
RNase A (2.3 Units/µl)	500µl	2.5ml	10ml	2-8°C
Collection Tubes (Spin Column)	50	250	1000	RT
Collection Tubes (Shredder)	50	250	1000	RT
Hand Book	1	1	1	–
Certificate of Quality	1	1	1	–

9. Specifications

Maximum amount of Plant Tissue	100mg wet weight 20mg dry weight
Capacity of column reservoir	700µl
Binding capacity of membrane (ds DNA)	50µg
Minimum Elution volume	50µl

10. How to Begin

A. Sample collection and storage

Do's

1. For obtaining best results, the starting material should be either fresh or has been immediately frozen in nitrogen and stored at – 80° C. Ground tissue powder can also be stored at –80° C.
2. After harvesting, the tissue should be dried or lyophilized for storage at room temperature.
3. For high DNA quality, samples should be dried within 24 hours after collection.
4. For higher yields, young samples should be collected as they contain more cells per weight and smaller amount of polysaccharides and polyphenolics making handling easier.

Don'ts

1. Do not subject the stored samples to repeated freezing and thawing as it leads to reduced DNA size.
2. Poor-quality starting material leads to reduced length and yield of purified DNA.

B. Maximum Amount of Starting Material

Do's

For dried tissues, reduce the maximum amount of starting material.

Don'ts

Do not exceed the recommended maximum amount of starting material, as it will result in inefficient lysis, leading to low yields and purity.

C. Clearing Lysate with mdi Shredder

Do's

1. It is recommended to remove cell debris and salt precipitates during gDNA isolation procedure, as it can lead to clogging of spin column.
2. For particulate matter forming a compact pellet, it is recommended to use mdi shredder spin column , remove all the debris and precipitates making the preparation of cleared lysate rapid and efficient.

D. Highly Viscous Samples

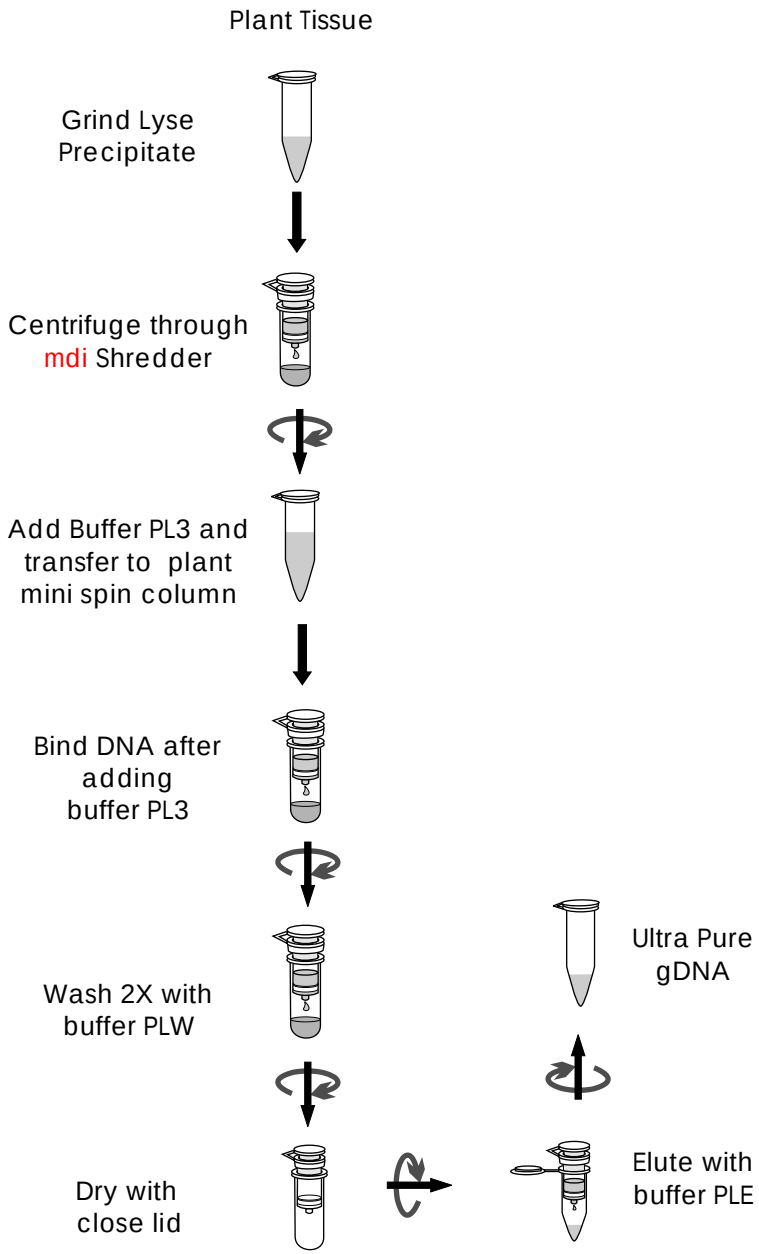
Do's

For lysate involved in layer formation, it is recommended to perform an additional centrifugation step.

Don'ts

Do not load highly viscous lysate and large amount of precipitates on the spin column, as the centrifugation of entire lysate through spin column can result in sheared DNA.

11. mdi Plant gDNA Miniprep Procedure



12. gDNA Isolation from Plant Tissue

12.1 Principle

Obtaining highly pure gDNA from plant tissue using mdi Plant gDNA Miniprep Kit involves:

Grinding and Lysis of Plant Tissue

To efficient lyse the plant tissue, grind it well in liquid nitrogen before addition of PL1 and RNase A.

Capturing of gDNA on mdi shredder Spin Column

In order to facilitate adsorption of gDNA onto the spin column, suitable conditions of salt concentration and pH are required, which is achieved by addition of binding buffer PL3.

Washing

Subsequent to DNA binding, unwanted components like proteins, carbohydrates and polysaccharides are washed away.

Washing is done by buffer PLW.

Elution

Salt concentration and pH of elution buffer is important for elution efficiency. Elution occurs at basic conditions and low salt concentration. Elution is performed with buffer PLE.

12.2 Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Maximum amount of plant tissue should be 100mg wet weight or 20mg dry weight.

Centrifugation:

1. All centrifugation steps should be carried out at room temperature at ≥ 10,000 rpm.
2. In case of choking of spin column, increase centrifugation time.

Lysis

1. For efficient lysis, grind the tissue completely into powder. To reduce lysis time, grind the sample in liquid nitrogen before addition of buffer PL1.
2. Add RNaseA to the sample before addition of buffer PL2 for complete removal of RNA.
3. In case of any precipitation in buffers, re-dissolve by warming to 37°C for few minutes.

Washing

1. To remove residual wash buffer, spin the column with close lid for 1 minute at ≥ 10,000 rpm.

2. Flow through should be properly discarded before centrifugation otherwise residual buffer will not be removed and may inhibit enzymatic reactions.

Elution

1. Elution buffer must be dispensed on to the center of column. For maximum elution efficiency, incubation time should be increased by 2-3 minutes.
2. For obtaining highly concentrated gDNA, elution should be done in two successive steps with buffer PLE in separate micro centrifuge tubes.

12.3 Protocol

1. Grind the plant material (Maximum 100 mg wet or 20 mg dried weight) under liquid nitrogen to a fine powder using a mortar and pestle. Transfer the powder and liquid nitrogen to an appropriately sized micro centrifuge tube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw, proceed immediately to the next step.

   Note: If liquid Nitrogen is not available then simply grind the plant material with mortar and pestle after adding 400µl buffer PL1, transfer it to an appropriately sized microcentrifuge tube. Then add 10µl of RNase A solution from stock (2.3 units/µl), mix well. Proceed to step 3.

2. Add 400µl of buffer PL1 and 10µl RNase A solution from stock (2.3 units/µl), mix by vortexing.

3. Incubate the mixture for 10 minutes at 65 °C in an oven or water bath. Mix 2-3 times during incubation by inverting the tube.

4. Add 130 µl of Buffer PL2, to the lysate mix gently but throughly by inverting the tube 5-6 times and incubate the mixture on ice for 5 minutes.

5. Centrifuge the lysate at ≥ 10,000rpm for 5 minutes.

6. Remove the supernatant carefully and apply it to the mdi shredder mini spin column placed in a 2ml collection tube and centrifuge for 2 minutes at ≥10,000 rpm.

7. Transfer the supernatant of the flow through without disturbing the pellet from the above step to a new micro centrifuge tube.

8. Add 1.5 volumes of buffer PL3 to the cleared lysate and mix by pipetting .

   For example, to 450µl lysate add 675µl buffer PL3.

9. Place the plant mini spin column in a collection tube and pass the above mixture through the spin column by centrifuging it at ≥10,000 rpm for 1 minute. Discard the flowthrough.

Note: The maximum volume of column reservoir is 700 µl. For sample volumes greater than 700 µl, simply load the remaining sample, balance the micro centrifuge and spin again. Discard the flow through.

10. Place the plant mini spin column in the same collection tube. Wash the spin column with 500 µl of buffer PLW by giving a spin at ≥ 10,000 rpm for 1 minute. Discard the flow through.

11. Again add 500µl of buffer PLW to the plant mini spin column and centrifuge for 1 minute at ≥ 10,000 rpm. Discard the flow through.

12. Place the spin column with close lid in the same collection tube and centrifuge at ≥ 10,000 rpm for 1 minute.

    Important: This step is necessary to remove residual wash buffer.

13. Place the plant mini spin column in a fresh 1.5 ml micro centrifuge tube (not provided).

14. Elute the bound gDNA in 100 µl of buffer PLE (users can elute the gDNA in a minimum 50µl volume if they wish to increase the concentration of gDNA) by adding the buffer directly to the center of the column and let it stand for 5 minutes. Spin at ≥ 10,000 rpm for 1 minute.

    Note: Repeat step 14 once more by taking a new micro centrifuge tube to increase the total recovery of gDNA.

13. Trouble Shooting Guide

A. Little or no Yield of gDNA

1. Overloading of spin column	The wet plant sample should not weigh more than 100mg and the dry plant sample not more than 20mg.
2. Incorrect lysate preparation	Check buffer volumes and ensure that incubation at 65 °C is not less than 10 minutes for proper cell lysis. Also for proper precipitation of proteins, incubation on ice should not be less than 5 minutes after adding buffer PL2. Check buffer PL1 for precipitates. Re-dissolve by warming to 37 °C.
3. Spin column choked	The column can choke in case the lysate is not clear before loading. Increase centrifugation time to obtain clear lysate.
4. Insufficient disruption of plant sample	Grind the plant material to a fine powder under liquid nitrogen, no tissue clump should remain.
5. Improper dispensing of elution buffer	The elution buffer must be dispensed properly on to the center of the column membrane.
6. Insufficient incubation of elution buffer in the column membrane	Increase incubation time by 2-3 minutes.

B: Low quality DNA

1. Nuclease contamination	Use autoclaved plastic and glassware.
2. RNA contamination	RNase digestion is insufficient. Check that RNase A is added to the lysate.
3. Sheared Genomic DNA	Avoid vigorous mixing.

C: DNA does not perform well

Residual wash buffer in eluate	Spin the column with close lid for 1-2 minutes extra at ≥10,000 rpm, to remove residual wash buffer completely.

14. Product Use Limitations

mdi kits are developed and manufactured for research purpose only. The products are not recommended to be used for human, diagnostics or drug purposes for which these should be cleared by the concerned regulatory bodies in the country of use.

15. Product Warranty and Satisfaction Guarantee

All mdi products are guaranteed and are backed by our

a. Technical expertise and experience of over 30 years.

b. Special mdi process for consistency and repeatability.

c. Strict quality control and quality assurance regimen.

d. Certificate of analysis accompanied with each product.

mdi provides an unconditional guarantee to replace the kit if it does not perform for any reasons other than misuse. However, the user needs to validate the performance of the kit for its specific use.

16. Ordering Information

To order please specify as below:

Type		XX	XX	XX	X	Pack Size	
Type	Code					Pack Size	Code
PTGK	PTGK					50	0050
						250	0250
						1000	1000

Example:

PTGK	XX	XX	XX	X	0250

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