Express 96 Plant Miniprep gDNA Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

mdi Express 96 Plant Miniprep gDNA Kit is designed to have a fast, easy and economical isolation of upto 15µg of high purity gDNA from plant samples. The kit incorporates a uniquely formulated buffer PL1 to lyse the plant samples and fast vacuum technology to purify it. This technology does away with phenol extraction (associated with desalting) and ethanol precipitation (associated with anion exchange based purification).

2. Applications

1. Automated Fluorescent Sequencing
2. Radioactive Sequencing
3. Southern Blotting
4. Labelling
5. Cloning
6. Quantitative, Real-Time PCR
7. RAPD, AFLP, RFLP Analysis
8. Microsatellite Analysis
9. SNP Genotyping

3. Storage Conditions

mdi Express 96 Plant Miniprep gDNA Kit should be stored at room temperature. The kit is stable for one year at room temperature without showing any reduction in performance and quality. RNase A should be stored at 2-8°C.

For longer storage, the entire kit can be stored at 2-8°C. In case precipitates are observed in buffer, re-disolve all buffers before use at 65°C for few minutes.

4. Quality Assurance

The mdi Express 96 Plant Miniprep gDNA 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 Express 96 Plant Miniprep gDNA Kit.

6. Lot Release Criteria

Each lot of Express 96 Plant Miniprep gDNA 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 system especially to satisfy your needs.

We welcome your feedback to improve our products.

8. Kit Contents

Contents	Quantity	Storage Temperature
Express Prep Plate	6	RT
Buffer PL1	280ml	RT
Buffer PL2	90ml	RT
Buffer PL3	375ml	RT
Buffer PLW	1200ml	RT
Buffer PLE	130ml	RT
RNaseA (2.3 Units/µl)	1500µl	2-8 °C
Tape Pad (Sterile)	15	RT
Flat Bottom Block (Sterile)	12	RT
Elution Microtubes (Racked)	576	RT
Caps for Elution Microtubes	576	RT
Hand Book	1	–
Certificate of Quality	1	–

9. Specifications

Maximum amount of Plant Tissue	50mg wet weight 10mg dry weight
Capacity of column reservoir	1ml
Binding capacity of membrane (ds DNA)	50µg
Minimum Elution volume	100-200µl

10. Volumes for a Miniprep

Plant Tissue Weight	50mg
Buffer PL 1	400µl
Buffer PL 2	130µl
Buffer PL 3	Approx. 600µl
Buffer PLW	1800µl
Buffer PLE	100µl

11. Principle

Obtaining highly pure gDNA from plant tissue using mdi Express 96 Plant Miniprep gDNA Kit involves:

Grinding and Lysis of Plant Tissue

To efficient lyse the plant tissue, grind it well after addition of buffer PL1.

Capturing of gDNA on Membrane

In order to facilitate adsorption of gDNA onto the membrane, 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. 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

Do's

1. It is recommended to remove cell debris and salt precipitates during gDNA isolation procedure, as it can lead to clogging of prep plate.

2. For particulate matter forming a compact pellet, it is recommended to centrifuge the lysate to 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 prep plate, as entire lysate passing through membrane can result in sheared DNA.

13. Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Maximum amount of plant tissue should be 50mg wet weight or 10mg dry weight.

Centrifugation:

1. All centrifugation steps should be carried out at room temperature.
2. In case of choking of membrane, increase vacuum.

Lysis

1. For efficient lysis, grind the tissue completely. For better quality completely grind the sample after 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 65°C for few minutes.

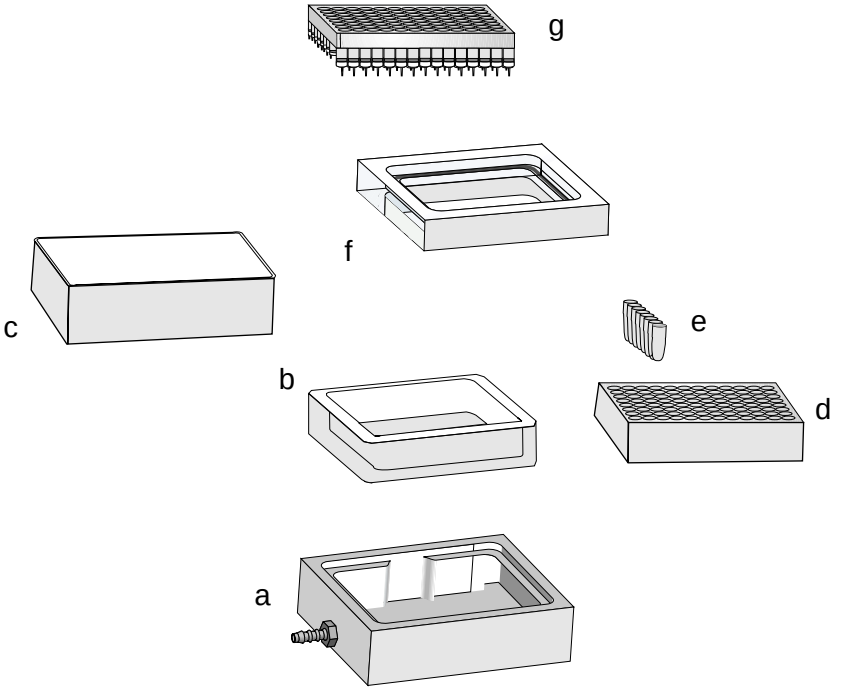
Washing

1. To remove residual wash buffer, apply additional vacuum for 10 minutes.

Elution

1. Elution buffer must be dispensed on to the center of membrane. 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 elution micro tubes.

14. mdi **Vacuum Manifold for 96 Well Purification Kits (VM 96)**



mdi VM 96 Manifold Components

a. mdi VM Base

b. Plate Holder

c. Waste Tray

d. Microtube Rack

e. Microtubes

f. mdi VM 96 Top Plate

g. Express Prep Plate

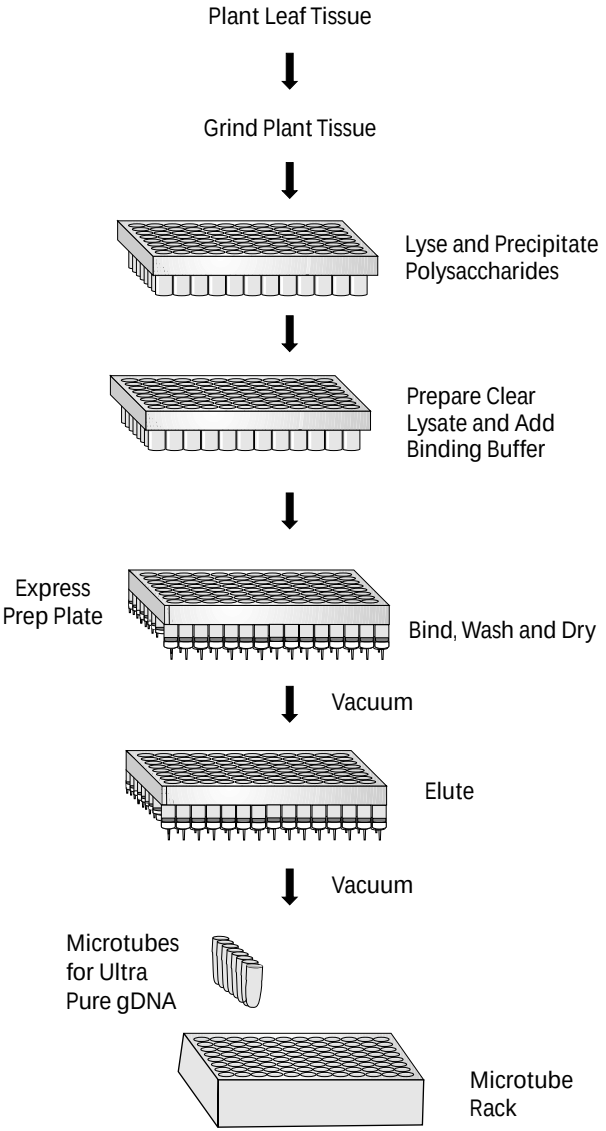
15. Guidelines for mdi (VM 96) Manifolds

1. mdi VM 96 manifold is operated with a house vacuum or Vacuum Pump
2. Always store mdi VM 96 manifolds clean and dry. To clean, simply rinse all components with water and dry with paper towels. Do not use solvents.
3. The components of mdi VM 96 manifold are not resistant to ethanol , methanol ,or other organic solvents. If solvents are spilled on the unit, rinse thoroughly with distilled water. Ensure that no residual Buffer PLW should be left in the mdi VM 96 manifold.

Things to be provided by the User

1. mdi VM 96 manifold
2. Multichannel Pipettes
3. Reservoir for multichannel pipettes
4. Centrifuge for micro titer plate
5. Vacuum Source
6. Vacuum Regulator

16. mdi Express 96 Plant gDNA Miniprep Procedure



17. Protocol

This protocol is designed for high-throughput plant gDNA minipreps using express prep plates on mdi VM 96. The kit accommodates upto 96 parallel preparation of up to 15 µg of ultra pure gDNA from plant material.

Note:

a. Do not add RNAse A in buffer PL1 directly.

b. If precipitates are observed in buffers, keep at 65 °C for some time.

Prepare mdi VM 96:

c. Place the Express prep plate in the mdi VM 96 top plate, make sure that the plate is seated securely. Seal unused wells of the Express prep plate with tape.

d. Place the waste tray inside of the mdi VM base.

e. Place mdi VM 96 top plate squarely over base. The express prep plate should now be placed above waste tray. Attach mdi VM to a vacuum source.

f. Regulate (-30 to -150) mm Hg vacuum on empty module using 3 way valve on vacuum regulator on mdi VM 96 before starting the procedure.

Procedure:

1. Grind the plant material (maximum 50mg wet or 10 mg dried weight). Using mortar and pestle after adding 400µl buffer PL1, then transfer each sample to wells of flat bottom block.

   Note: Alternatively plant samples can be ground using other methods.

2. Add 2.5µl RNAse A of (2.3 units/µl) into each sample.

3. Mix by pipetting up and down and seal the block with the tape.

4. Incubate the flat bottom block at 65 °C in an oven for 10 minutes.

5. Remove and discard tape. Add 130 ì l Buffer PL2 to each sample.

6. Mix by pipetting up and down. Seal the wells with tape ensure that the wells are properly sealed to avoid leakage.

7. Incubate the flat bottom block for 10 minutes at –20°C. This incubation aids the precipitation of proteins and inhibitors of downstream applications following addition of Buffer PL2.

8. Centrifuge the flat bottom block for 5 min at 2500-3000 rpm. Compact pellets will form, but some particles may float. Be careful not to transfer any of these particles in the following step.

9. Remove and discard the tape. Carefully transfer (approx. 400 ì l) each supernatant to new flat bottom block (provided).

10. Add 1.5 volumes (approx. 600 ì l) of Buffer PL3 to each sample. A white precipitate may form upon addition of Buffer PL3. This precipitate does not interfere with the procedure and subsequent application.

11. Mix by pipetting up and down.

12. Carefully transfer the sample to each well of the prep plate. Take care not to wet the rims of the wells to avoid aerosols. Unused wells of the prep plate should be sealed with tape. Apply vacuum until all samples have passed through.

    The optimal flow rate is approximately 1-2 drops/second which can be regulated by using a 3-way valve or vacuum regulator between the mdi VM 96 and the vacuum source. The flow through is collected in the waste tray.

13. Switch off vacuum. Wash express prep plate by adding 900µl buffer PLW to each well and applying vacuum. Repeat wash step once more.

14. After buffer PLW has been drawn through all wells, apply maximum vacuum for an additional 10 minutes to dry the membrane.

    Important: Apply maximum vacuum to dry the membrane. Turn off vacuum regulator or leakage valves if they are used. This step is necessary to remove residual wash buffer. Residual buffer may inhibit subsequent down stream applications.

15. Switch off vacuum, and ventilate the mdi VM 96 slowly lift the top plate from the base (do not lift the express prep plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the express prep plate with clean absorbent paper.

16. For elution into provided elution microtubes:

    Replace waste tray with the elution microtube rack containing elution microtubes. Place the top plate back on the base, making sure that the express prep plate is seated securely.

17. To elute DNA, add 100µl of buffer PLE to the center of each well of the express prep plate, let stand for 1 minute and apply maximum vacuum for 5 minutes, switch off vacuum and ventilate mdi VM 96 slowly.

18. Repeat step 17 with another 100µl buffer PLE for higher yield.

18. Trouble Shooting Guide

A. Little or no Yield of gDNA

1. Overloading of prep plate	The wet plant sample should not weigh more than 50mg and the dry plant sample not more than 10mg.
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 at -20°C should not be less than 10 minutes after adding buffer PL2. Check buffer PL1 for precipitates. Re-dissolve by warming to 65 °C.
3. 96 well membrane choked	The wells 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 using suitable method, no tissue clump should remain.
5. Improper dispensing of elution buffer	The elution buffer must be dispensed properly on to the center of the membrane.
6. Insufficient incubation of elution buffer in the 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	Apply maximum vacuum for 10 minutes to remove residual wash buffer completely.

19. 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.

20. 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 quality 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.

21. Ordering Information

To order please specify as below:

Type		XX	XX	XX	X	Pack Size	
Type	Code					Pack Size	Code
EPGK	EPGK					6	0006

Example:

EPGK	XX	XX	XX	X	0006

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