Endotoxin Free pDNA Miniprep Kit

User Guide

mdi
Membrane Technologies

ADVANCED MICRODEVICES PVT. LTD.
21, Industrial Area, Ambala Cantt - 133006 (INDIA)
Tel: 0171-2699290, 2699471, 2699274
Fax: +91-171-2699221, 2699008
Email: mdi@vsnl.com
Website: www.mdimembrane.com

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1. Introduction

mdi Endotoxin Free pDNA Miniprep Kit is uniquely designed to facilitate ultrapure pDNA free from endotoxins (<0.1EU/µg), ideal for transfection even into highly sensitive mammalian cells. These kits offer high yields from both low copy as well as high copy number plasmid. The innovative buffer system and filter device provides efficient binding of pDNA onto the spin column.

Washing is done with the help of provided wash buffers in order to remove endotoxins, RNA, proteins and polysaccharides. The technology also does away with hasselsome gravitational waiting, phenol extraction (associated with desalting) and ethanol precipitation (associated with anion exchange based purification).

2. Downstream Applications

1. Automated Fluorescent Sequencing
2. Radioactive Sequencing
3. Restriction Digestion
4. Transfection (with highly sensitive mammalian cell lines)
5. Cloning
6. PCR

3. Storage Conditions

mdi Endotoxin Free pDNA Miniprep 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.

For longer storage, the entire kit can be stored at 2-8°C. In case precipitates are observed in buffer, re-dissolve all buffers before use at 37°C for few minutes. All buffers should be at room temperature before starting the protocol.

Important

After adding RNase A to buffer M1, it should be stored at 2-8°C and is stable for 6 months.

In case of any precipitation, re-dissolve the buffers by warming to 37°C.

4. Quality Assurance

The **mdi** Endotoxin Free pDNA 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 a lab coat, disposable gloves and protective goggles while working with the **mdi** Endotoxin Free pDNA Miniprep Kit.

6. Lot Release Criteria

Each lot of **mdi** Endotoxin Free pDNA 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 offers 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

Pack Content	Quantity			Storage Temperature
mdi Endotoxin Free Mini Spin Columns	50	250	1000	RT
Collection Tube	50	250	1000	RT
Buffer M1	15ml	60ml	240ml	RT
Buffer M2	15ml	60ml	240ml	RT
Buffer M3	15ml	60ml	240ml	RT
Buffer MB	15ml	60ml	240ml	RT
Buffer EF	15ml	60ml	240ml	RT
Buffer MPW	30ml	150ml	600ml	RT
Buffer MW	50ml	225ml	900ml	RT
Buffer ME	20ml	100ml	400ml	RT
RNase A (2.3 units/µl)	40µl	150µl	600µl	2-8°C
Handbook	1	1	1	- -
Certificate of Quality	1	1	1	- -

Note: After adding RNase A, buffer M1 should be stored at 2-8°C.

9. Specifications

Features	High Yield Protocol	
Type of Plasmid	High Copy Number Plasmid	Low Copy Number Plasmid
Capacity of Column Reservoir (µl)	800	800
Binding Capacity of Spin Column (µg)	≥ 25	≥ 25
Maximum Culture Volume (ml)	3 - 5	5
Expected (µg) Yield of Plasmid	≥ 20	≤ 20

10. Principle

The **mdi** Endotoxin Free pDNA Miniprep Kit allows the isolation of ultra pure pDNA which involves:

1. Lysis and Neutralization of Bacterial Culture
2. Capturing pDNA on spin column
3. Removal of Endotoxins
4. Washing
5. Elution

1. Lysis and Neutralization of Bacterial Culture

To efficiently lyse the bacterial culture, centrifuge it properly before addition of buffer 'M1' & 'M2'. The lysed culture is then neutralized with the help of buffer 'M3'.

2. Capturing pDNA on Spin Column

In order to facilitate adsorption of pDNA onto the spin columns, suitable conditions of salt concentration and pH are required which is achieved by addition of binding buffer 'MB'.

3. Removal of Endotoxins

Wash with buffer 'EF' to remove bacterial endotoxins and to achieve <0.1 EU (Endotoxin Units) per µg of plasmid DNA in the final eluate.

4. Washing

Subsequent to pDNA binding onto the spin column, unwanted components like RNA, proteins and polysaccharides are washed away. Washing is done by buffer 'MPW' & 'MW'.

5. Elution

Salt concentration and pH of elution buffer is important for elution efficiency, elution occurs at basic conditions and low salt concentration. Elution is done with buffer 'ME'.

11. Important Points to be Considered

Optimization of Operating Conditions

All parameters regarding pDNA yield needs to be monitored like plasmid copy number, host strains, culture media, culture volume for obtaining expected high yields.

Centrifugation

All centrifugation steps should be carried out at room temperature at ≥ 10,000 rpm

In case of choking of spin column, increase centrifugation time.

Lysis

After adding RNase, buffer 'M1' should be stored at 2-8 °C.

Washing

To remove residual wash buffer, spin the column for 1-2 minute extra at ≥ 10,000 rpm.

Elution

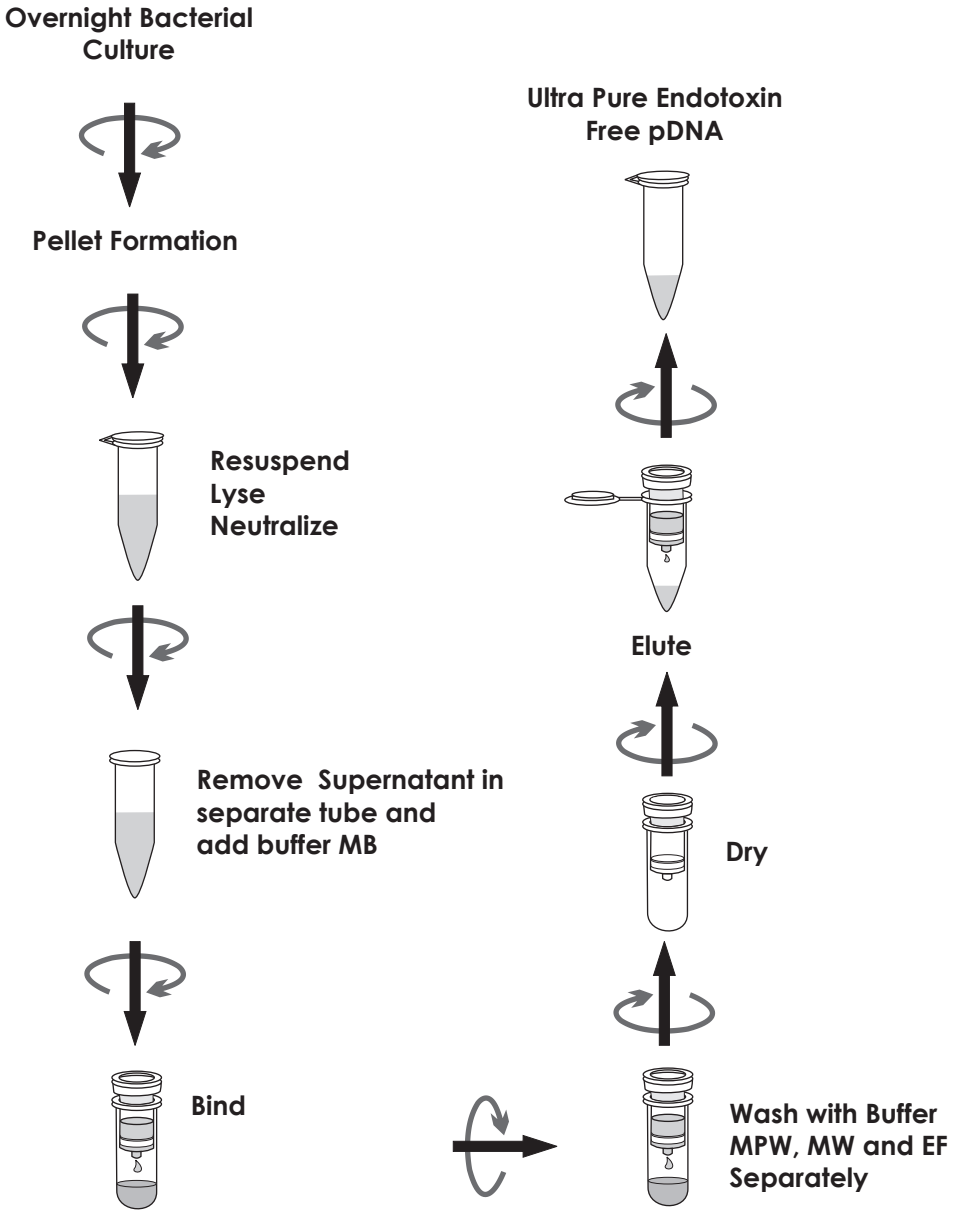
Elution buffer must be dispensed on to the center of the spin column for maximum elution efficiency, incubation time should be increased by 2-3 minutes.

For obtaining highly concentrated pDNA, elution should be done in low salt concentration buffer 'ME'.

Yield

pDNA yield can be determined by spectrophotometer at 260nm and by Agarose gel electrophoresis. Purity is detected by A_{260}/A_{280} ratio lying between 1.8-2.0.

12. mdi Endotoxin Free pDNA Miniprep Procedure



13. Protocol

Plasmid DNA purification using mdi Endotoxin Free pDNA Miniprep Kit

Important: This protocol is designed for the preparation of upto 20µg of Endotoxin free (<0.1 EU/µg) plasmid DNA using the **mdi** Endotoxin Free pDNA Miniprep Kit with a maximum culture volume of 5ml.

Maximum recommended culture volumes:

Copy Number	High-Yield Protocol
High Copy Plasmid	3-5ml
Low Copy Plasmid	5ml

Procedure

Note: Add provided RNaseA in the buffer M1 before use by pipetting 1ml M1buffer into the RNase A vial, shake it, and then transfer the mixture into the M1 bottle. Store at 2-8°C and use within 6 months.

***If precipitates are observed in buffer M2, keep it at 37 °C for some time.**

1. Centrifuge 1-5ml of overnight bacterial culture at 10,000 rpm for 5 minutes. Remove all the traces of supernatant by inverting the open tube until all medium has been drained.

2. Resuspend the pellet in 200µl of buffer M1 completely by vortexing or pipetting up and down and transfer to a 1.5ml micro centrifuge tube.

   Ensure that RNaseA has been added to buffer M1.

   No cell clumps should be visible after resuspension.

3. Add 200µl of buffer M2 , mix gently but thoroughly by inverting the tube 4-6 times.

   Mix gently by inverting the tube .Do not vortex ,as this may result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 2 minutes.

Bottle containing buffer M2 should be closed immediately after use to avoid acidification from CO_2 in the air.

4. Add 200 µl of buffer M3, mix immediately but gently by inverting the tube 4-6 times.

5. Centrifuge at 13,000 rpm for 10 minutes. A compact white pellet will form.

   Note: Increase centrifugation time to 15 minutes at 10,000 rpm

6. Collect the supernatant quickly and carefully in separate microcentrifuge tube and add 200 µl of buffer MB. **Then incubate at room temperature for 5 minutes.**

7. Place the spin column in the collection tube. Transfer the sample to the spin column and pass through the spin column by giving a spin at ≥ 10,000 rpm for 1 minute. Discard the flowthrough.

   Note: Maximum volume of the column reservoir is 800µl. For sample volumes >800µl, simply load the remaining sample, balance the micro centrifuge and spin again. Discard the flow through.

8. Place the spin column in the same collection tube. Wash the miniprep spin column with 200µl of buffer EF by giving a spin at ≥10,000 rpm for 1 minute. Discard the flow through.

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

10. Place the spin column in the same collection tube. Wash the miniprep spin column with 750µl of buffer MW by giving a spin at ≥10,000 rpm for 1 minute. Discard the flow through.

11. Place the spin column in same collection tube and centrifuge for an additional 1 minute at ≥10,000rpm.

    Important: This step is necessary to remove residual wash buffer. Residual wash buffer will not be completely removed unless the flow through is discarded before this additional centrifugation. Residual wash buffer may inhibit subsequent enzymatic reactions.

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

13. Elute the bound pDNA in a minimum of 50µl of buffer E (users can elute the pDNA in a greater volume if they wish to) by adding the buffer directly to the center of the column membrane and let it stand for 1 minute. Spin at ≥10,000 rpm for 1 minute.

14. Trouble Shooting Guide

A. Little or no DNA

1. Plasmid did not propagate	Please check that the conditions for optimal culture growth were met.
2. Poor bacterial growth	Inoculate from a freshly streaked plate and incubate in a shaker after inoculation.
3. Precipitates in BufferM2	Redissolve by warming to 37 °C.
4. Cell resuspension incomplete	The bacterial pellet should be evenly resuspended in buffer M1. Do not add buffer M2 until an even suspension is achieved.
5. Poor cell lysis	Too many cells harvested from an overgrown culture. Use culture of proper O.D. grown in not more than 16 hours.
6. Improper dispensing of the elution buffer	The elution buffer must be dispensed properly on to the center of the column membrane.

B: Low quality DNA

1. Nuclease contamination	Use autoclaved plastic and glass wares.
2. RNA contamination	Rnase digestion is insufficient. Check that RNase A is added to buffer M1. Also if buffer M1 is more than 6 months old, add more RNase A.
3. Genomic DNA in eluate	Avoid excessive vortexing or vigorous mixing.

4. Plasmid degradation	Do not incubate in M2 for more than prescribed time in step 3.
5. Incomplete drying	Results in solvent in the eluate which is difficult to load in gel, also results in reduced yield and difficulties in digestion. Spin the column for 2-3 minutes extra at $\geq$10,000 rpm to completely remove the solvent.

15. 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.

16. 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.

17. Ordering Information

To order please specify as below:

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

Example:	EFPK	XX	X	XX	XX	0250

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