Endotoxin Free Quanta Mega Kit

User Guide

Membrane Technologies

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1. Introduction

mdi Endotoxin Free Quanta Mega Kit is uniquely designed to facilitate ultrapure pDNA (2.5 mg) 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 plasmids. 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 Quanta Mega 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 buffer by warming to 37 °C

4. Quality Assurance

mdi Endotoxin Free Quanta Mega 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 **mdi** Endotoxin Free Quanta Mega Kit.

6. Lot Release Criteria

Each lot of **mdi** Endotoxin Free Quanta Mega 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 offer 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 Size	Quanta Mega Kit- 2	Storage Temperature
mdi Quanta Mega Spin Columns	2	RT
mdi Quanta Mega Filter Device (with Green Adaptor)	2	RT
Tube Extender	2	RT
mdi Green Adaptor (with black holder) for 45mm Neck Bottle	2	RT
45mm Neck Bottle* (1000ml)	2	RT
Buffer M1	25 x 2 ml	RT
Buffer M2	25 x 2 ml	RT
Buffer M3	25 x 2 ml	RT
Buffer MB	25 x 2 ml	RT
Buffer EF	4 x 2 ml	RT
Buffer MPW	25 x 2 ml	RT
Buffer MW	25 x 2 ml	RT
Buffer ME	10 x 2 ml	RT
RNase A (2.3 units/µl)	100 x 2 µl	2-8 °C
Handbook	1	- -
Certificate of Quality	1	- -

* To be arranged by the user.

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 Tube Extender (ml)	300	300
Binding Capacity of Spin Column (µg)	3000	3000
Maximum Culture Volume (ml)	500	500
Expected (µg) Yield of Plasmid	upto 2500	upto 2500

10. Principle

The **mdi** Endotoxin Free Quanta Mega 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 ≥ 8,000 rpm

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

Lysis

After adding buffer M2 invert 4-6 times and incubate at room temperature for 5 minutes.

Washing

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

Elution

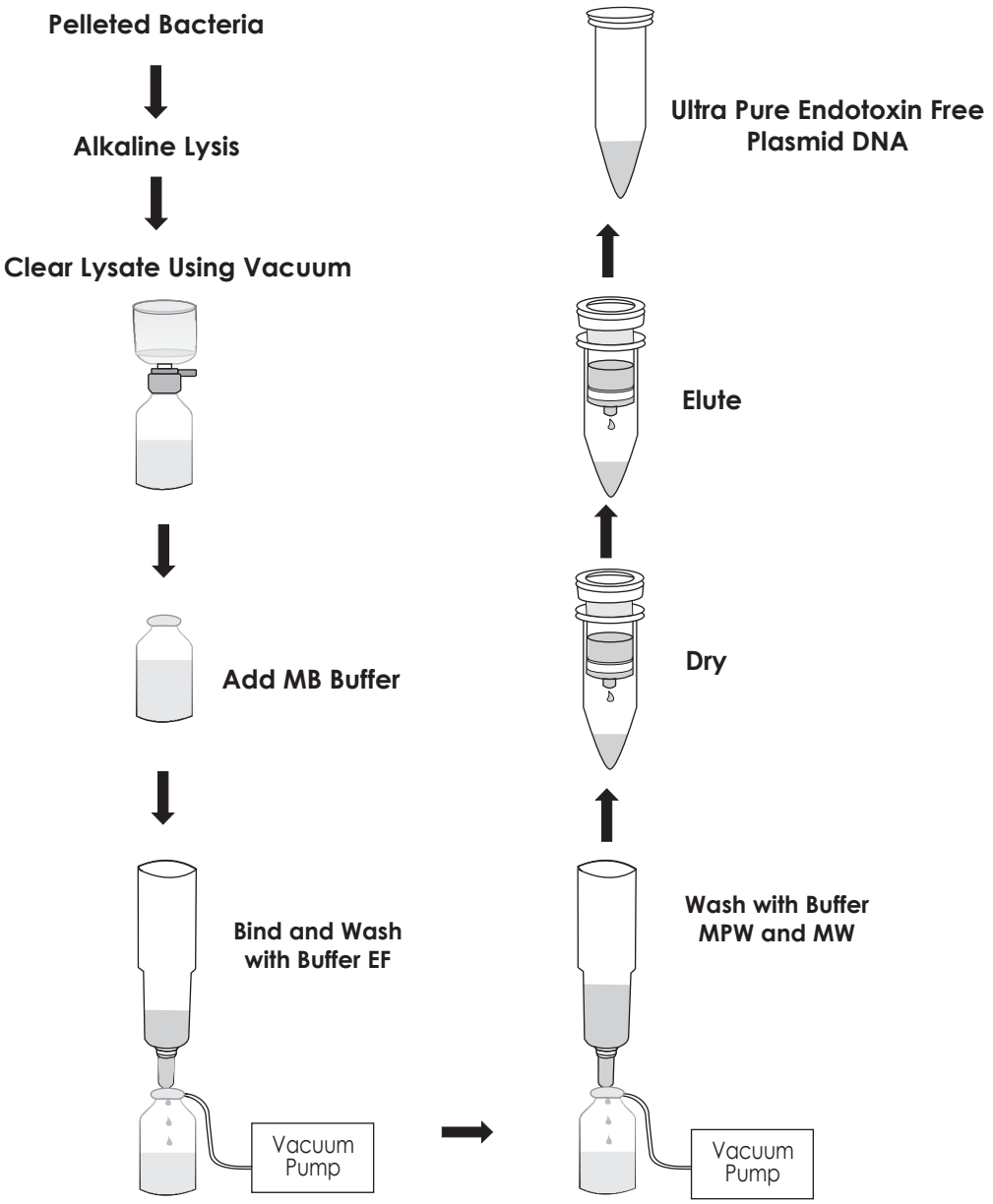
Elution buffer must be dispensed on to the center of the spin column for maximum elution efficiency. Incubation time should be increased by 2-3 minutes.

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 Quanta Mega Procedure



13. Protocol

Plasmid DNA purification using mdi Endotoxin Free Quanta Mega Kit

Important: This protocol is designed for the preparation of upto 2.5 mg of Endotoxin free (<0.1 EU/µg) plasmid DNA using the **mdi** Endotoxin Free Quanta Mega Kit from high copy as well as low copy number plasmid with a maximum culture volume of 500 ml or a pellet wet weight of 2.5gm from fermentation cultures.

1. **Ensure that RNase A has been added to buffer 'M1' before starting the protocol**
2. **Do not pipette out or measure the buffers while adding in different steps of protocol, just pour whole the buffers from the respective bottles except buffer ME bottles.**

Procedure:

1. Centrifuge 500ml of bacterial cultures from high copy number or low copy number plasmids in LB medium of O.D. 2.5 - 3.5 at 600nm at 8000 rpm for 15 minutes or take a pellet wet weight of 2.5 gms from fermentation cultures.
2. Resuspend the pelleted bacteria in 25 ml of buffer M1.

   Note: For efficient lysis, it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
3. Pour 25ml of Buffer M2. Gently mix by inverting 4-6 times and incubate at room temperature for 5 minutes.

   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 5 minutes.

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

4. During incubation attach the **mdi** filter device onto a 45mm neck bottle of capacity of 1,000ml and connect to a vacuum source.
5. Pour 25ml of buffer M3 to the lysate, mix immediately by inverting 4-6 times. Proceed directly to the next step. Do not incubate lysate on ice.
6. Pour the lysate into the **mdi** filter device attached on to a 45mm neck bottle. **Incubate at room temperature for 10 minutes.**

   Do not disturb the **mdi** Mega filter device during incubation. A precipitate containing proteins, genomic DNA and detergent will float and form a layer on the top of the lysate. This ensures convenient filtration without clogging. If the precipitate has not floated to the top of the lysate after incubation then carefully run a sterile pipet tip around the walls of the **mdi** Mega filter device.
7. Switch on the vacuum source and apply vacuum till all liquid has been drawn through the **mdi** Mega filter device. Apply approx-300 mmHg vacuum. (The recovered volume is approx. 60-65ml
8. Remove **mdi** Quanta Mega filter device from the 45mm neck bottle and pour 25ml buffer MB, close the lid and mix by inverting 4-6 times.

   Incubate at room temperature for 5 minutes.
9. Attach the **mdi** Quanta Mega spin column with tube extender to the new 45mm neck bottle (capacity 1000 ml) with the help of "Green adaptor" (with black holder).
10. Transfer the lysate to the **mdi** Quanta Mega spin column and pass the lysate through the spin column by applying vacuum (approx - 300mmHg) using a vacuum source. For this attach vacuum source to the outlet of the green adaptor attached to the 45mm neck bottle.
11. To achieve extremely low endotoxin level, add 4 ml of buffer EF to the Quanta Mega spin column and apply vacuum to pass half of the volume. Switch off the vacuum pump, disconnect the tubing and incubate at room temperature for 5 minutes. Reconnect the vacuum source and again apply vacuum to pass the remaining buffer through the column.

12. Wash the **mdi** Quanta Mega spin column with 25ml of buffer MPW by applying vacuum.
13. Wash the **mdi** Quanta Mega spin column with 25ml of buffer MW by applying vacuum.
14. Remove **mdi** Quanta Mega spin column from the "Green adaptor" and also remove tube extender from the **mdi** Quanta Mega spin column.
15. Place the **mdi** Quanta Mega spin column in a sterile 50ml centrifuge tube and centrifuge for 5minutes at ≥ 8,000 rpm.

    Important: This spin is necessary to remove residual wash buffer.
16. Place the **mdi** Quanta Mega spin column in a new sterile DNase free 50ml centrifuge tube. For maximum concentration add 1 ml of buffer ME to the center of spin column, (for maximum recovery add 2ml of buffer ME) let it stand for at least 5 minutes and then centrifuge for 5 minutes at 8,000 rpm.
17. Reload the above eluate in the same **mdi** Quanta Mega spin column, incubate for 5 minutes and elute in the same microcentrifuge tube by centrifuging at ≥ 10,000 rpm for 5 minutes.

    (Repeat step 17 once more to get high concentration of pDNA. Average eluate volume is 0.6ml from 1ml and 1.5ml from 2ml).

14. Trouble Shooting Guide

A. Poor or no DNA Yield

1. Plasmid did not propagate	Please check that the conditions for optimal culture growth were met.
2. Poor bacterial growth	Inoculate the culture under optimum conditions and ensure that all conditions are adequately met.
3. Lysis was not efficient	If larger than recommended culture volume was used or cell density was very high (usually occurs if the culture is grown more than 16 hours). Reduce the culture volume and use culture grown between 12-16 hours.
4. Buffer M2 and MB Precipitated	Redissolve by warming to 37°C.
5. Insufficient cell resuspension	The bacterial pellet formed after 15 minutes centrifugation should be resuspended completely in buffer 'M1' by pipetting up and down.

6. Column was overloaded	Can happen if larger than recommended culture volumes are used.
7. Improper dispensing of elution buffer	The elution buffer must be dispensed properly onto the center of the column membrane for maximum elution efficiency. Increase incubation time by 2-3 minutes.

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'. If buffer 'M1' is older than 6 months, add more RNaseA.
3. Genomic DNA in eluate	Avoid excessive vortexing or vigorous mixing.
4. Plasmid Degradation	Do not incubate in buffer 'M2' for more than the prescribed time.

C: DNA does not perform well

1. Residual wash buffer in eluate	Spin the column for 2-3 minutes extra at $\geq$ 10,000 rpm to remove residual wash buffer completely.

D: mdi Filter Device Clogs During Filtration of Lysate

1. Too large culture volume used	Do not exceed the culture volume recommended in the protocol.
2. Inefficient mixing after addition of buffer 'M3'	Mix well until a fluffy white material has formed.
3. Mixing too vigorous after addition of buffer 'M3'	After addition of buffer 'M3', the lysate should be mixed immediately but gently. Vigorous mixing disrupts the precipitate into tiny particles which may clog the mdi filter devices.
4. Lysate was not loaded immediately into the mdi filter device after addition or mixing of buffer 'M3'	Load the lysate immediately after addition and mixing of buffer 'M3'. Decanting after incubation may disrupt the precipitate into tiny particles which may clog the mdi filter device.
5. Old pellet was used	Use fresh pellet.

E: mdi Quanta Mega Spin Column Choked

1. Lysate was not clear after filtration through mdi filter device	Do not apply extreme vacuum force as it may push tiny particles into the downstream of mdi filter device.

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	XX	XX	X	Pack Size	
Type	Code					Pack Size	Code
QMEK	QMEK					2	0002
						10	0010

Example:

QMEK	XX	XX	XX	X	0010

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