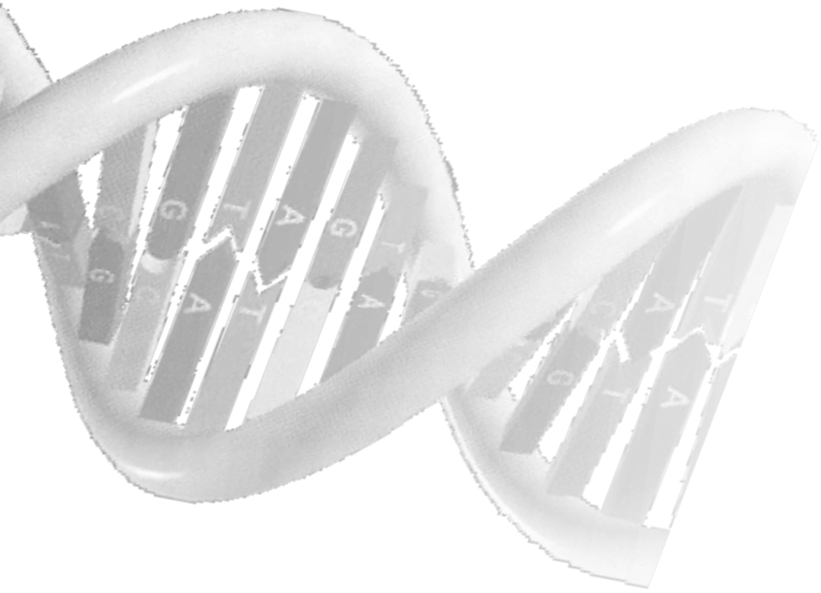
Quanta Midi Kit



User Guide

Membrane Technologies

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1. Introduction

mdi Quanta Midi Kit is uniquely designed to facilitate high yield, ultrapure pDNA from both low copy as well as high copy number plasmid. The innovative buffer system reagents 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 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
5. Cloning
6. PCR

3. Storage Conditions

mdi Quanta Midi 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** Quanta Midi 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 Quanta Midi Kit.

6. Lot Release Criteria

Each lot of Quanta Midi 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 Size	Quanta Midi Kit-25	Quanta Midi Kit-100	Storage Temperature
mdi Quanta Midi Spin Columns	25	100	RT
mdi Quanta Midi Filter Device (with Red Stopper)	25	100	RT
Tube Extender	25	100	RT
mdi Green Adaptor for 45mm Neck Bottle	1	1	RT
45mm Neck Bottle* (250 or 500ml)	1	1	RT
Collection Tube	25	100	RT
Buffer M1	160ml	650ml	RT
Buffer M2	160ml	650ml	RT
Buffer M3	160ml	650ml	RT
Buffer MB	160ml	650ml	RT
Buffer MPW	160ml	650ml	RT
Buffer MW	30ml	120ml	RT
Buffer ME	15ml	60ml	RT
RNase A (2.3 units/µl)	640µl	2.6ml	2-8 °C
Handbook	1	1	–
Certificate of Quality	1	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)	35	35
Binding Capacity of Spin Column (µg)	350	350
Maximum Culture Volume (ml)	25-35	50
Expected (µg) Yield of Plasmid	150-250	30-100

10. Principle

The **mdi** Quanta Midi kit allows the isolation of high ultra pure pDNA which involves:

1. Lysis and Neutralization of Bacterial Culture
2. Capturing pDNA on spin column
3. Washing
4. 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. **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'.

4. **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 buffer 'M2' invert 4-6 times and incubate at room temperature for 3 minutes.

Washing

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

Elution

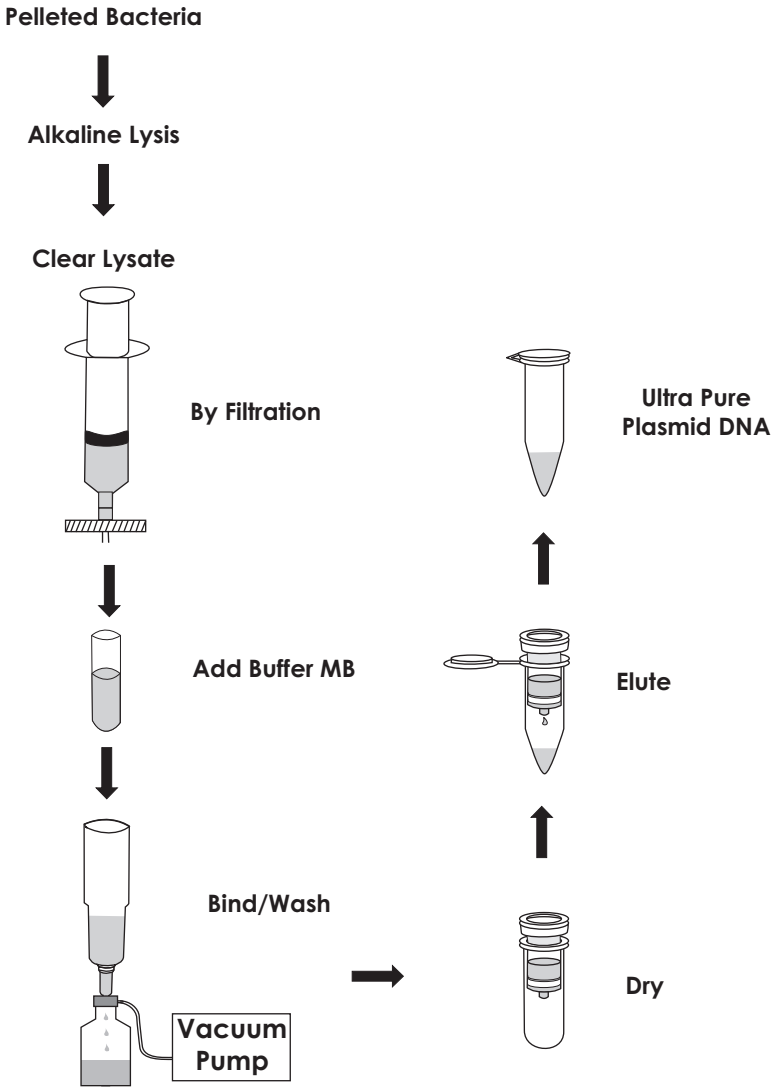
Elution buffer must be dispensed on to the center of the spin column for maximum elution efficiency, incubation time should be Increased by 2-3 minutes.

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

Yield

pDNA Yield can be determined by both 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 Quanta Midi Procedure



13. Protocol

Plasmid DNA purification using mdi Quanta Midi Kit

Important: This protocol is designed for the preperation of upto 250µg high -copy plasmid DNA or low copy plasmid DNA using the **mdi** Quanta Midi Kit with a maximum culture volume of 50ml.

Maximum recommended culture volumes:

Copy Number	High-Yield Protocol
High Copy Plasmid	25-35ml
Low Copy Plasmid	50ml

Ensure that RNase A has been added to buffer 'M1' before starting the protocol.

Procedure:

1. Centrifuge 25 - 35ml bacterial culture for high copy number plasmids and 50ml for low copy number plasmids of OD_{600nm} 2.5 - 3.5 at 8,000 rpm for 15 minutes.
2. Resuspend pelleted bacteria in buffer M1 (4ml for high copy number plasmids and 6ml for low copy number plasmids).

   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. Add buffer 'M2' (4ml for high copy number plasmid and 6ml for low copy number plasmid). Gently mix by inverting 4-6 times and incubate at room temperature for 3 minutes.

   During incubation, prepare the **mdi** filter device with 'RED STOPPER'. Place the **mdi** filter device into a convenient tube or a rack, after attaching it to the outlet of the 20ml syringe without plunger.

4. Add buffer 'M3' (4ml for high copy number plasmid and 6ml for low copy number plasmid) to the above lysate mix immediately by inverting 4-6 times. Proceed directly to next step. Do not incubate lysate on ice.

5. Pour the lysate into the barrel of the 20ml syringe with the **mdi** filter device attached to it. **Incubate at room temperature for 5 minutes.**

6. Remove the 'RED STOPPER' from the **mdi** filter device outlet nozzel. Gently insert the plunger into the 20ml syringe having lysate and filter the cell lysate into a new sterile tube allowing space for the addition of buffer 'MB'.

   Filter until all the lysate has passed through the **mdi** filter device, but do not apply extreme force as it may push debris through the filter and choke it. (Approximately the amount of lysate that can be recovered after filtration through the **mdi** filter device is 11 - 12ml for high copy number plasmids and 17 - 18ml for low copy number plasmids).

7. Add buffer MB (4ml for high copy number plasmid and 6ml for low copy number plasmid) to the cleared lysate and mix by inverting 4-6 times. **Incubate at room temperature for 5 minutes.**

8. Attach the **mdi** Quanta Midi spin column with tube extender to the 45mm neck bottle with the help of 'GREEN ADAPTOR'.

9. Transfer the lysate to the **mdi** Quanta Midi spin column and pass the lysate through the **mdi** Midi Spin Column by applying vacuum (approx - 300mmHg) using a vacuum pump. For this, attach the vacuum pump to the outlet of the green adaptor attached to the 45mm neck bottle.

   Note: Switch off vacuum pump when all the sample has passed through the spin column.

10. Wash the **mdi** Quanta Midi spin column with buffer 'MPW' (4ml for high copy number plasmid and 6ml for low copy number plasmid) by applying vacuum.
11. Remove the tube extender and wash the **mdi** Quanta Midi spin column with 750µl of buffer 'MW' by applying vacuum.
12. Remove **mdi** Quanta Midi spin column from the 'GREEN ADAPTOR'.
13. Place the **mdi** Quanta Midi spin column in a 2ml collection tube (provided) and centrifuge for 1 minute at ≥10,000 rpm.

    Important: This spin is necessary to remove residual wash buffer.
14. Place the **mdi** Quanta Midi spin column in a fresh 1.5ml microfuge tube (not provided). To elute the DNA, add 200µl of buffer ME to the center of the spin column, let it stand for at least 1 min and centrifuge for 1 minute at 10,000 rpm.
15. **Optional:** For higher concentration. Reload above eluate in the same **mdi** Quanta Midi spin column, incubate for 1 minute and elute in the same microfuge tube by centrifuging at ≥ 10,000 rpm for 1 minute.

    (Repeat step 15 once more to get high concentration of pDNA. Average eluate volume is 190µl from 200µl.)

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 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 is 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. Insufficient lysis for low copy plasmid	For low copy plasmid preparations, increasing the volumes of buffers M1, M2, M3, MB and MPW by 2ml may help to increase plasmid yield and quality.
5. Buffer M2 and MB precipitated	Redissolve by warming to 37°C.
6. Insufficient cell resuspension	The bacterial pellet formed after 15 minutes centrifugation should be resuspended completely in buffer 'M1' by pipetting up and down.

7. Column was overloaded	Can happen if larger than recommended culture volumes are used.
8. Improper dispensing of elution buffer	The elution buffer must be dispensed properly onto the center of 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 vigrous mixing.
4. Plasmid Degradation	Do not incubate in buffer M2 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 barrel of syringe having attached 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.
6. Extreme force was applied during filtration	Do not apply extreme force as it may push tiny particles into the mdi filter device.

E: mdi Quanta Midi Spin Column Choked

1. Lysate was not clear after filtration through mdi filter device and takes very long to pass through the column	Incubate the lysate for 5-10 minutes after adding buffer M3. After precipitates are visible on top, gently filter the clear lysate. Do not apply extreme force as it may push tiny particles through the 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
QDPK	QDPK					25	0025
						100	0100

Example:

QDPK	XX	XX	XX	X	0100

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