# Endotoxin Free Quanta Midi Kit

## User Guide

Membrane Technologies

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## 1. Introduction

**mdi** Endotoxin Free Quanta Midi 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 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** Endotoxin Free 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 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 Quanta Midi Kit.

## 6. Lot Release Criteria

Each lot of **mdi** Endotoxin Free 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 |
| Collection Tube | 25 | 100 | RT |
| Buffer M1 | 160 ml | 650 ml | RT |
| Buffer M2 | 160 ml | 650 ml | RT |
| Buffer M3 | 160 ml | 650 ml | RT |
| Buffer MB | 160 ml | 650 ml | RT |
| Buffer EF | 25 ml | 90 ml | RT |
| Buffer MPW | 160 ml | 650 ml | RT |
| Buffer MW | 30 ml | 120 ml | RT |
| Buffer ME | 15 ml | 60 ml | RT |
| RNase A (2.3 units/µl) | 640 µl | 2.6 ml | 2-8 °C |
| Handbook | 1 | 1 | – |
| Certificate of Quality | 1 | 1 | – |

**Components to be arranged by the user**

| | | | |
|---|---|---|---|
| 45mm Neck Bottle(250 or 500ml) | 1 | 1 | RT |
| 20 mL Syringes | 25 | 100 | RT |
| 50 mL Sterile Tubes | 25 | 100 | RT |
| 1.5 mL Centrifuge Tubes | 25 | 100 | RT |

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** Endotoxin Free Quanta Midi 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 performed 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 performed 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 $\geq$ 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 $\geq$ 10,000 rpm.

**Elution**

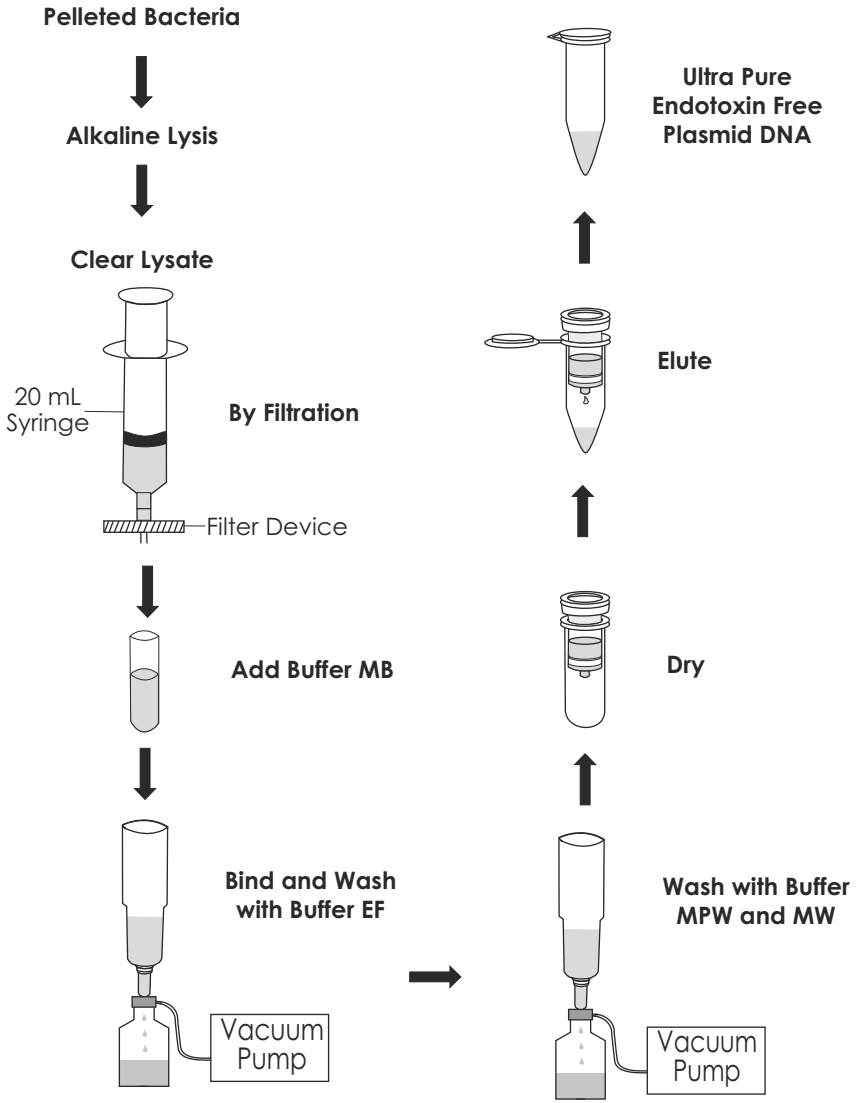
Elution buffer must be dispensed on to the center of the spin column for maximum elution efficiency, incubation time should be increased by 2-3 minutes.

For obtaining highly concentrated pDNA, elution should be performed 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 Midi Procedure



## 13. Protocol

**Plasmid DNA purification using mdi Endotoxin Free Quanta Midi Kit**

**Important:** This protocol is designed for the preparation of upto 250 µg of Endotoxin free (<0.1 EU/µg) plasmid DNA using the **mdi** Endotoxin Free Quanta Midi Kit with a maximum culture volume of 50 ml.

**Maximum recommended culture volumes:**

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

**Please ensure that:**

1) RNase A has been added to buffer 'M1' before starting the protocol.

2) 20 mL syringe is ready for use

3) The GREEN ADAPTER is fixed on the 45 mm neck bottle and ready for use

**Procedure:**

1. Centrifuge 25 - 35 ml bacterial culture for high copy number plasmids and 50 ml for low copy number plasmids of $OD_{600nm}$ 2.5 - 3.5 at 8,000 rpm for 15 minutes.

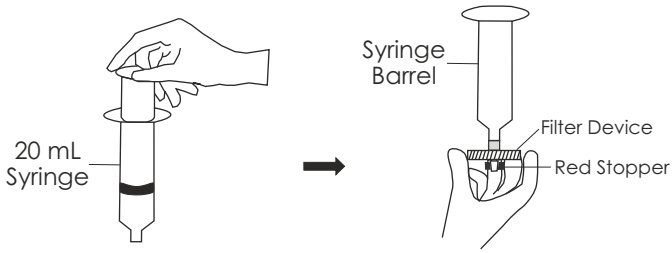
2. Resuspend pelleted bacteria in buffer M1 (4 ml for high copy number plasmids and 6 ml for low copy number plasmids).

   **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' (4 ml for high copy number plasmids and 6 ml for low copy number plasmids). 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'. Remove the plunger from the 20 mL syringe and attach the **mdi** filter device to its outlet. Do not remove the RED STOPPER.

   Place this setup on a convenient tube or a rack.

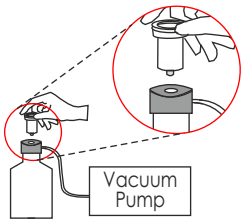



Remove the plunger from the syringe

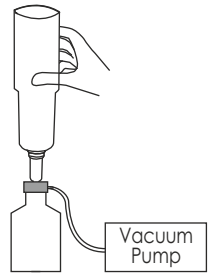
Attach the **mdi** filter device to syringe barrel outlet

4. Add buffer 'M3' (4 ml for high copy number plasmids and 6 ml for low copy number plasmids) 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 20 ml 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. Gently insert the plunger into the 20 ml syringe having lysate and filter the cell lysate into a new sterile 50 ml 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 volume of lysate that can be recovered after filtration through the **mdi** filter device is 11 - 12 ml for high copy number plasmids and 17 - 18 ml for low copy number plasmids).
7. Add buffer 'MB' (4 ml for high copy number plasmids and 6 ml for low copy number plasmids) 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 to the 'GREEN ADAPTOR' fitted on the 45 mm neck bottle. Fit the tube extender on the spin column.




Attach the spin column to GREEN ADAPTOR fitted on the 45 mm neck bottle




Fit the tube extender on the spin column

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

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

10. To achieve extremely low endotoxin level, add 800 µl of buffer EF to the Quanta Midi 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.

11. Wash the **mdi** Quanta Midi spin column with buffer 'MPW' (4 ml for high copy number plasmids and 6 ml for low copy number plasmids) by applying vacuum.

12. Remove the tube extender and wash the **mdi** Quanta Midi spin column with 750 µl of buffer 'MW' by applying vacuum.

13. Remove **mdi** Quanta Midi spin column from the 'GREEN ADAPTOR'.

14. Place the **mdi** Quanta Midi spin column in a 2 ml collection tube (provided) and centrifuge for 1 minute at ≥10,000 rpm.

    **Important: This spin is necessary to remove residual wash buffer.**

15. Place the **mdi** Quanta Midi spin column in a fresh 1.5 ml microcentrifuge tube (not provided). To elute the DNA, add 200 µl 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.

16. **Optional:** For higher concentration. Reload the above eluate in the same **mdi** Quanta Midi spin column, incubate for 1 minute and elute in the same microcentrifuge tube by centrifuging at ≥ 10,000 rpm for 1 minute.

    (Repeat step 16 once more to get high concentration of pDNA. Average eluate volume is 190 µl from 200 µl.)

## 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 was very high (usually occurs if the culture is grown more than 16 hours).<br><br>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 2 ml 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 the column membrane for maximum elution efficiency.<br><br>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 is 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 | X | XX | XX | Pack Size | |
|---|---|---|---|---|---|---|---|
| Type | Code | | | | | Pack Size | Code |
| QDEK | QDEK | | | | | 25 | 0025 |
| | | | | | | 100 | 0100 |

| Example: | QDEK | XX | X | XX | XX | 0100 |
|---|---|---|---|---|---|---|

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