Bacterial gDNA Miniprep Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

mdi Bacterial gDNA Miniprep Kit is a fast, economical and easy isolation method of high purity gDNA from Bacteria. The buffer system provided in the kit allows Bacteria lysis followed by selective binding of gDNA to the spin column. This Kit does away with lysing enzymes and consequently consuming incubation time. It also does not require any bead based lysing systems.

Purified gDNA is eluted in low-salt buffer or water for variety of downstream applications. This technology does away with the cumbersome methodologies of phenol extraction as well as ethanol precipitation.

2. Applications

1. Automated Fluorescent Sequencing
2. Radioactive Sequencing
3. Restriction Digestion
4. Cloning
5. PCR

3. Storage Conditions

Optimum storage conditions at which all components of the kit can be preserved without alteration in it's quality and performance.

One Year	RT
Longer Storage	2-8°C

Important

In case of any precipitation observed in the buffers, re-dissolve by warming at 37°C for sometime and cool it down to room temperature.

4. Quality Assurance

The **mdi** Bacterial gDNA 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: 2015 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 Bacterial gDNA Miniprep Kit.

6. Lot Release Criteria

Each lot of Bacterial gDNA 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 customize products for special applications. We will

* Simulate problems, and suggest alternative methods to solve them.
* Make changes/ improvements in our existing products/protocols.
* Develop special new products and systems especially to satisfy your needs.

We welcome your feedback to improve our products.
Please write to info@mdimembrane.com

8. Kit Contents

Contents	Quantity			Storage Temperature
Spin Columns	50	250	1000	RT
mdi Shredder Spin Columns	50	250	1000	RT
Collection Tubes (Spin Column)	50	250	1000	RT
Collection Tubes (Shredder)	50	250	1000	RT
Buffer CB	55ml	275ml	1100ml	RT
Buffer Wash-1	30ml	150ml	600ml	RT
Buffer Wash-2	30ml	150ml	600ml	RT
Buffer E	20ml	100ml	400ml	RT
Hand Book	1	1	1	–
Certificate of Quality	1	1	1	–

9. Specifications

Maximum amount of Bacteria pellets	50mg - 100 mg
Maximum Number of Bacteria cell	10^9
Capacity of column reservoir	700µl
Binding capacity of membrane (ds DNA)	50µg
Minimum Elution volume	50µl

10. 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 stored at – 20° C.
2. Sample source can be Gram negative or Gram positive bacteria
3. Sample should be estimated by pellet weight
4. Pellet should be prepared at ≥ 10,000 rpm
5. Pellet should be resuspended completely

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

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 with mdi Shredder

Do's

1. It is recommended to remove cell debris and salt precipitates during gDNA isolation procedure, as it can lead to clogging of spin column.
2. For particulate matter forming a compact pellet, it is recommended to use **mdi** shredder spin column to remove all the debris and precipitates making the preparation of cleared lysate rapid and efficient.

D. Highly Viscous Samples

Do's

It is recommended to perform an additional centrifugation step.

Don'ts

Do not load large amount of highly viscous lysate on the spin column, as the centrifugation of entire lysate through spin column can result in sheared DNA.

E. Typical gDNA yields from Bacterial Cells

Sample	O.D	Pellet Weight	Total DNA (µg)
Bacillus subtilis	3.34	54mg	35.95
XL-1 Blue	3.71	52mg	26.4
Mycobacterium smegmatis	3.15	50mg	11.4

11. mdi gDNA Miniprep Procedure

Bacterial Cell

Lyse

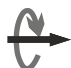
Shredder

Bind

Wash 2X

Dry

Elute



Ultra Pure gDNA

12. gDNA Isolation from Bacteria

12.1 Principle

Obtaining highly pure gDNA from bacterial cells using **mdi** gDNA Miniprep Kit involves:

Lysis of bacterial cells

For efficient lysis resuspend bacterial pellet completely in buffer CB and vortex for 10 min at high speed.

Clearing Lysate with mdi Shredder

1. It is recommended to remove cell debris and salt precipitates during gDNA isolation procedure, as it can lead to clogging of spin column.
2. For particulate matter forming a compact pellet, it is recommended to use **mdi** shredder spin column to remove all the debris and precipitates making the preparation of cleared lysate rapid and efficient.

Capturing of gDNA on Spin Column

In order to facilitate adsorption of gDNA onto the spin column, suitable conditions of salt concentration and pH are required, which is achieved by addition of buffer CB.

Washing

Subsequent to DNA binding, unwanted components like proteins, carbohydrates and cell debris are washed away. Washing is performed by buffer wash 1 and Wash 2.

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 E.

12.2 Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Maximum number of bacterial cells should be 10^9 or wet pellet weight 50mg - 100 mg.

Centrifugation:

1. All centrifugation steps should be carried out at room temperature at $\geq$ 10,000 rpm.
2. In case of choking of spin column, increase centrifugation time.

Lysis

1. For efficient lysis resuspend bacterial pellet completely and vortex for 10 minutes at high speed.
2. In case of any precipitation in buffers, re-dissolve by warming to 37°C for few minutes.
3. Add 5µl of ß-mercaptoethanol per 1ml buffer CB and mix well.

   Note: ß-mercaptoethanol is not provided

Washing

1. To remove residual wash buffer, spin the column lid for 1 minute at $\geq$ 10,000 rpm.
2. Flowthrough should be properly discarded before centrifugation otherwise residual buffer will not be removed and may inhibit enzymatic reactions.

Elution

1. Elution buffer must be dispensed on to the center of column. 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 E in same micro centrifuge tubes by reloading eluate of first elution

12.3 Protocol

This protocol has been developed for purification of total gDNA from Gram negative and Gram Positive bacteria.

Procedure

1. Centrifuge bacterial culture(10^9 cells or 50 - 100 mg wet pellet) in a microcentrifuge tube at 10,000 rpm for 5 minutes. Remove all the traces of supernatant by inverting the open microcentrifuge tube until all medium has been drained.

2. Resuspend the pellet in 1ml of Buffer CB containing ß-mercaptoethanol (5µl/ml)completely by pipetting up and down.

3. Vortex for 10 minute at high speed.

4. Place the **mdi** shredder spin column in the collection tube and pass 500µl lysate through it by centrifuging at ≥10,000 rpm for 1 minute.

5. Place the spin column in the collection tube and remove the lysate from the shredder collection tube without disturbing the pellet that may form at bottom of collection tube. Load the lysate in the spin column. Pass the lysate by centrifuging at ≥ 10,000 rpm for 1 minute. Discard the flowthrough and place spin column in the same collection tube.

6. Again place the mdi shredder spin column(refer step-4) in the same collection tube and pass remaining lysate through it by centrifuging at ≥10,000 rpm for 1 minute

7. Remove the lysate from collection tube (step-6) without disturbing the pellet and pass it through the same spin column(step-5) placed in collection tube by centrifuging at ≥10,000rpm for 1 minute. Discard the flowthrough.

8. Place the spin column in the same collection tube Wash the column with 500µl of buffer Wash-1 by centrifuging for 1 minute at ≥10,000 rpm. Discard the flow through.

9. Again Place the spin column in the same collection tube and wash the column with 500µl of buffer Wash-2 by centrifuging for 1 minute at ≥10,000 rpm. Discard the flow through.

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

    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.

11. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided).

    Elute the bound gDNA by adding 100µl(minimum 50µl) of buffer E directly to the center of the column membrane and let it stand for 1 minute. Spin at ≥10,000 rpm for 1 minute.

12. Reload the eluate from step-11 in the same column wait for 1 minute then spin at ≥ 10,000 rpm for 1 minute.

13. Trouble Shooting Guide

A. Little or no Yield of gDNA

Problem	Solution
1. Overloading of spin column	Ensure optimum cell count and wet weight of bacterial pellet.
2. Precipitates in buffer	In case of any precipitates in buffers, re- dissolve by warming to 37°C before use.
3. Spin column choked	Increase centrifugation time while passing sample through the spin column.
4. Insufficient Lysis	Ensure 10 minutes vortexing at high speed. Increase vortexing for 5 minutes more.
5. Improper dispensing of elution buffer	The elution buffer must be dispensed properly on to the centre of the column membrane.
6. Insufficient incubation of elution buffer in the column membrane	Increase incubation time by 2-3 minutes.
7. Poor Quality Sample	Use fresh sample or sample stored at -20°C t o -70°C. Avoid repeated freezing and thawing of sample.

B: Low quality DNA

1. Nuclease contamination	Use autoclaved plastic and glassware.

C: DNA does not perform well

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

D: Low A_{260}/A_{280} ratio

DNA diluted with water instead of buffer	Use buffer E to dilute the sample before measuring A_{260}/A_{280} ratio for purity.

14. 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.

15. Product Warranty and Satisfaction Guarantee

All **mdi** products are guaranteed and are backed by our

a. Technical expertise and experience of over 40 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.

16. Ordering Information

To order please specify as below:

Type	XX	XX	XX	X	Pack Size

Type	Code
BDGK	BDGK

Pack Size	Code
50	0050
250	0250
1000	1000

Example:

BDGK	XX	XX	XX	X	0250

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UGLBDGKKIT1505A