

Medi G-M Blood Genomic DNA Microprep Kit



User Guide

mdi
Membrane Technologies

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1. Introduction

The mdi Medi G-M Blood Genomic DNA (gDNA) Microprep Kit is a fast, economical and easy isolation method of high purity gDNA from whole blood . The buffer system provided in the kit allows efficient lysis followed by selective binding of gDNA to the spin column.

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 (associated with slurries formation) as well as ethanol precipitation (associated with anion exchange based purification system) for desalting.

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 to 56°C for sometime and cool it down to room temperature.

4. Quality Assurance

The mdi Medi G-M Blood gDNA Microprep 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 Medi G-M Blood gDNA Microprep Kit.

6. Lot Release Criteria

Each lot of Medi G-M Blood gDNA Microprep 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

- * Stimulate 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.

8. Kit Contents

Contents	Quantity			Storage Temperature
	50	250	1000	
Spin Columns	50	250	1000	RT
Collection Tubes	50	250	1000	RT
Buffer BL	17ml	85ml	340ml	RT
Buffer BW1	30ml	150ml	600ml	RT
Buffer BW2	30ml	150ml	600ml	RT
Buffer BE	22ml	110ml	440ml	RT
Proteinase K	1.25ml	6ml	24ml	2-8°C
Hand Book	1	1	1	--
Certificate of Quality	1	1	1	--

Note:

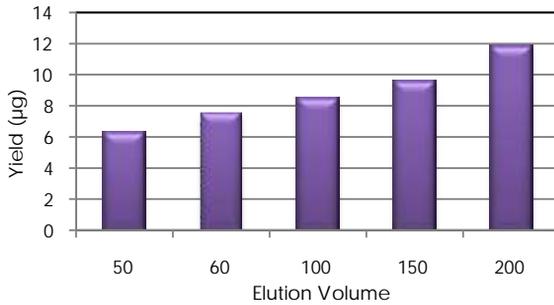
1. Proteinase K is provided in the kit. 1ml of Proteinase K (20mg/ml or 600mAU/ml) is required for 50 spin columns.
2. RNase A is not provided in the kit. 200µl of RNase A (100mg/ml or 7 units/µl) or 500µl of RNase (2.3 units/µl) is required for 50 spin columns.
3. RNase A (2.3 units/µl) can be purchased from [mdi](#) separately.

9. Specifications

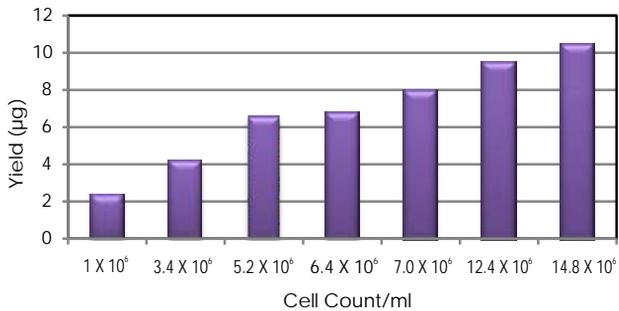
Maximum Volume of Whole Blood Sample	200µl
Capacity of column reservoir	700µl
Binding capacity of membrane (ds DNA)	10µg
Elution volume	60 - 200µl

10. Performance Parameters (Whole Blood)

A. Elution Volume vs Yield (Leucocyte Count = $7.1 \times 10^6/\text{ml}$)



B. Leucocyte Count vs Yield (Elution Volume = 60µl)



11. How to Begin

A. Sample collection and storage

Do's

For obtaining best results, the starting material should be either fresh or that has been immediately frozen and stored at -20 °C or -70 °C.

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

Do's

Maximum starting sample should be 200µl.

Don'ts

Overloading of **mdi** spin columns lead to significantly lower yields than expected.

C. Copurification of RNA

mdi Medi G-M Blood gDNA Microprep Kit copurifies RNA when present in sample. RNA inhibits some downstream enzymatic reactions.

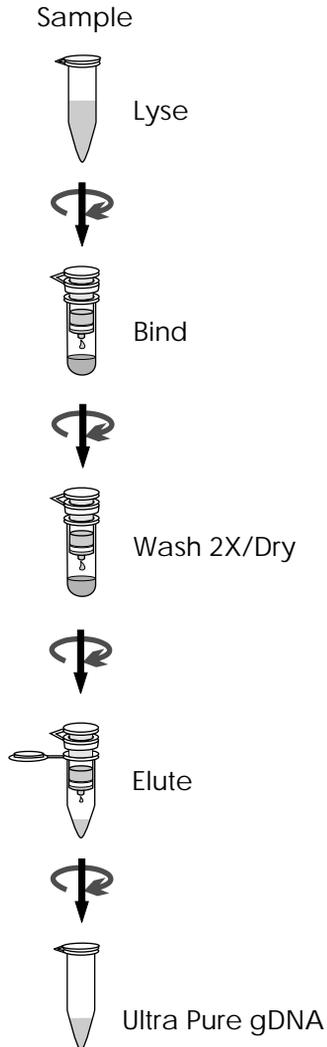
In order to get RNA free gDNA:

1. RNase A should be added to the sample before addition of buffer BL for RNA digestion
2. **mdi** Medi G-M Blood gDNA Microprep Kit recommends the use of RNase A stock solution(100mg/ml or 7 units/µl or 2.3 units/µl).
3. RNase amounts are to be adjusted with less concentrated stock solutions but its amount should not exceed 20 µl.

D. Elution

With **mdi** Medi G-M Blood gDNA kit, highly concentrated gDNA is obtained with 60µl of pre-heated (at 70 - 75°C for 30 minutes in an oven)elution buffer BE.

12. mdi Medi G-M Blood gDNA Microprep Procedure



13. Medi G-M gDNA Isolation from Blood

13.1 Principle

Obtaining highly pure gDNA from blood using **md**i Medi G-M Blood gDNA Microprep Kit involves:

Lysis of Blood Sample

For efficient lysis, mix blood sample completely with Proteinase K and buffer BL, then incubate at 56°C.

Capturing of gDNA on Spin Column

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

Washing

Subsequent to gDNA binding, unwanted components like proteins, carbohydrates and cell debris are washed away.

Washing is performed with buffer BW1 and BW2.

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 'BE', pre-heated at 70-75°C for 30 minutes in an oven. For obtaining highly concentrated gDNA, perform elution with 60µl of buffer BE.

13.2 Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Maximum amount of blood sample should be 200 μ l.

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, in case of whole blood, mix blood sample completely with Proteinase K and buffer BL, then incubate at 56°C.
2. Add RNaseA to the sample before addition of buffer BL for complete removal of RNA.
3. Proteinase K should be added to sample before addition of buffer BL to inactivate nuclease that can degrade DNA during purification.
4. In case of any precipitation in buffer BL, re-dissolve by warming to 56°C for few minutes.

Washing

1. To remove residual wash buffer, spin the column with closed lid for 3 minute at $\geq 10,000$ rpm.

Elution

1. Use pre-heated (at 70 - 75°C for 30 minutes in an oven) buffer BE.
2. Elution buffer must be dispensed on to the center of column. For maximum elution efficiency, incubation time should be increased by 5-6 minutes.
3. For obtaining highly concentrated gDNA, perform elution with 60 μ l of buffer BE.

13.3 Protocol (DNA Purification from Whole Blood)

Note: Things to do before starting

1. Heat a water bath at 56°C
2. Arrange ethanol (96-100%)
3. Preheat the elution buffer BE at 70-75°C for 30 minutes in an oven

Procedure:

1. Pipet 20µl Proteinase K into the bottom of a 1.5ml microcentrifuge tube.
2. Add 200µl blood sample to the microcentrifuge tube and mix well by pipetting up and down.

Note: It is possible to add Proteinase K to the sample that have already been dispensed into microcentrifuge tube.

3. Add 4µl of RNase A from 100mg/ml stock or 28 units and mix well by pipetting up and down. Use 10µl of RNase A from stock (2.3 units/µl).
4. Add 300µl buffer BL to the sample, mix throughly by vortexing.

Note: Do not add Proteinase K directly to buffer 'BL'.

5. Incubate at 56°C in water bath for 15 minutes. Mix 2-3 times by inverting the tube during incubation.
6. Briefly centrifuge the sample after incubation to remove drops from inside of lid.
7. Add 200µl Ethanol, mix well by vortexing and briefly centrifuge the sample to remove drops from inside of lid.
8. Place the spin column in the collection tube, pass the above sample through the spin column by spinning at $\geq 10,000$ rpm for 1 minute. Discard the flow through.

Note: Maximum volume of the column reservoir is 700µl. For sample volumes $>700\mu\text{l}$, simply load remaining sample balance and spin at $\geq 10,000$ rpm for 1 minute. Discard the flow through. If the sample does not pass after 1 minute then centrifuge for additional 1 minute.

9. Place the spin column in the same collection tube. Wash the column with 500µl of buffer BW1 by centrifuging for 1 minute at $\geq 10,000$ rpm. Discard the flowthrough.
10. Place the spin column in the same collection tube. Wash the column with 500µl of buffer BW2 by centrifuging for 3 minute at $\geq 10,000$ rpm. Discard the flowthrough, and collection tube.
11. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided)
12. Elute the bound gDNA by adding 60µl of pre-heated buffer BE directly to the center of the column membrane and incubate it at room temperature for 5 minutes. Spin at $\geq 10,000$ rpm for 1 minute.

User may elute with 200µl of buffer BE for maximum yield.

Use only buffer BE for elution as elution in water may result in acid hydrolysis of DNA over long term storage.

14. Trouble Shooting Guide

A. Little or no Yield of gDNA

- | | |
|--|---|
| 1. Overloading of spin column | Can happen due to very high cell (Leukocyte) density, as in case of infected donors. |
| 2. Precipitates in buffer | In case of any precipitates in buffers, re- dissolve by warming to 56°C before use. |
| 3. Spin column choked | Increase centrifugation time while passing sample through the spin column. |
| 4. Insufficient Lysis | Ensure incubation temperature of 56 °C. Check that Proteinase K was added to the sample. |
| 5. Improper dispensing of elution buffer | The elution buffer must be dispensed properly on to the center of the column membrane. |
| 6. Insufficient incubation of elution buffer in the column membrane | Increase incubation time by 2-3 minutes. |
| 7. Insufficient mixing of sample with buffer BL and ethanol before loading on to the spin column | Add buffer BL to the sample first and mix by vortexing. Then after incubation for 15 minutes add ethanol to the sample and again mix by vortexing. |
| 8. Poor Quality Sample | Use fresh sample or sample stored at -20°C to -70°C. Avoid repeated freezing and thawing of sample. |
| 9. Improper storage of blood samples | For short term storage blood samples should be stored at 4°C in tubes containing a standard anticoagulant (E.D.T.A.). For long term, store at -70°C with anticoagulant. |

B: Low quality DNA

- | | |
|---------------------------|---|
| 1. Nuclease contamination | Use autoclaved plastic and glassware. |
| 2. RNA contamination | RNase digestion is insufficient. Check that RNase A is added to the lysate. |
| 3. Sheared Genomic DNA | Avoid vigorous mixing. |

C: DNA does not perform well

Residual wash buffer in eluate	After washing with buffer BW2, discard the flowthrough and place the spin column in the same collection tube with closed lid. Centrifuge for additional 2-3 minutes at $\geq 10,000$ rpm.
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D: Low A_{260}/A_{280} ratio

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

High level of residual RNA	RNase A treatment should be performed as per the protocol.
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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	P	Pack Size	
Type	Code					Pack Size	Code
BRGK	BRGK					50	0050
						250	0250
						1000	1000

Example:

BRGK	XX	XX	XX	P	0250
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