Medi G

Blood Genomic DNA Miniprep Kit

User Guide

mdi
Membrane Technologies

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Table of Contents

1. Introduction

The mdi Medi G Blood Genomic DNA (gDNA) Miniprep Kit is a fast, economical and easy isolation method of high purity gDNA from 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 Blood 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: 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 Blood g DNA Miniprep Kit.

6. Lot Release Criteria

Each lot of Medi G Blood 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

* 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
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 A from 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 Blood Sample	200µl
Capacity of column reservoir	700µl
Binding capacity of membrane (ds DNA)	50µg
Elution volume	200µl

10. 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 Blood gDNA Miniprep Kit copurifies RNA when present in sample. RNA inhibits some downstream enzymatic reactions.

In order to get RNA free gDNA:

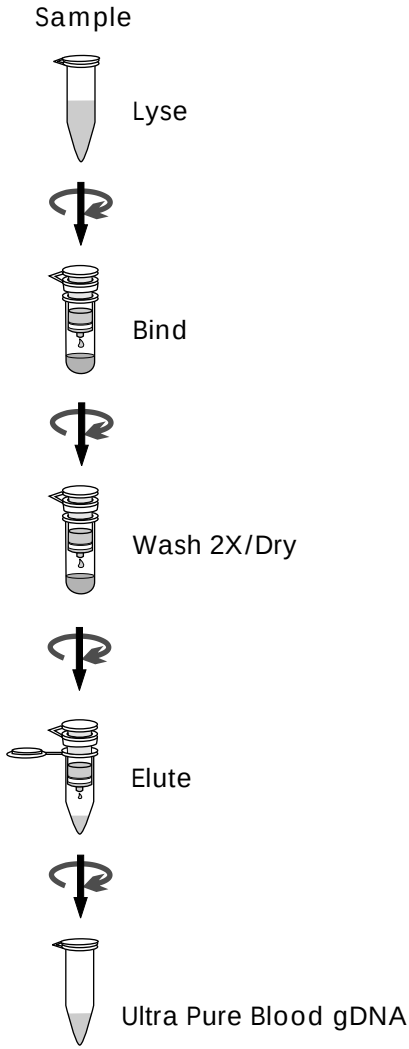
1. RNase A should be added to the sample before addition of buffer BL for RNA digestion
2. mdi Medi G Blood gDNA Miniprep Kit recommends the use of RNase A stock solution (100mg/ml or 7 units/µl or 2.3 units/µl).
3. RNase amounts are to be adjusted with less concentrated stock solutions but its amount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

D. Elution

With mdi Medi G Blood gDNA kit, highly concentrated gDNA is obtained with a 200µl of elution buffer BE, where eluate from the first elution is reloaded to maximize DNA concentration.

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

11. mdi Medi G Blood gDNA Miniprep Procedure



12. Medi G gDNA Isolation from Blood

12.1 Principle

Obtaining highly pure gDNA from blood using mdi Medi G Blood gDNA Miniprep 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 done by 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'.

12.2 Important Points to be Considered

Starting Material

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

Centrifugation:

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

Lysis

1. For efficient lysis, 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 ≥ 10,000 rpm.

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, elute with 50-100µl of buffer BE.

12.3 Protocol

Note: Things to do before starting

1. Heat a water bath at 56°C
2. Arrange ethanol (96-100%)

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 pipeting 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 >700µl, 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 ≥ 10,000rpm. 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 ≥ 10,000rpm. Discard the flowthrough, and collection tube.
11. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided)

    Elute the bound gDNA by adding 200µl of buffer BE directly to the center of the column membrane and let it stand for 2 minutes. Spin at ≥ 10,000rpm for 1 minute.
12. Reload above eluate in the same spin column. Incubate for 2 minutes and elute in the same microcentrifuge tube by centrifuging at ≥ 10,000rpm for 1 minute.

    Note: For highly concentrated gDNA elute with 50-100µl of buffer BE.

13. 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	Spin the column with closed lid for 3 minutes at $\geq$10,000 rpm after adding buffer BW2.

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.

E: High A_{260}/A_{280} ratio

High level of residual RNA	RNase A treatment should be performed as per the protocol.

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 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.

16. Ordering Information

To order please specify as below:

Type		XX	XX	XX	P	Pack Size	
Type	Code					Pack Size	Code
BMGK	BMGK					50	0050
						250	0250
						1000	1000

Example:

BMGK	XX	XX	XX	P	0250

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