Stool Genomic DNA Miniprep Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

The **mdi** Stool Genomic DNA (gDNA) Miniprep Kit is a fast, economical and easy isolation method of high purity gDNA from stool. The buffer system provided in the kit allows efficient lysis followed by selective binding of gDNA to the spin column.

Stool samples contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. Reagents and columns of the kit are developed in such a way that in combination they remove all inhibitory substances and increase the sensitivity of enzymatic reactions by thousand fold. Example: PCR, Restriction Digestion, etc.

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** Stool 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 Stool g DNA Miniprep Kit.

6. Lot Release Criteria

Each lot of Stool 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	200	1000	4000	RT
Buffer MSL	100ml	500ml	2000ml	RT
Buffer SB	33ml	165ml	660ml	RT
Buffer SW1	30ml	150ml	600ml	RT
Buffer SW2	30ml	150ml	600ml	RT
Buffer SE	20ml	100ml	400ml	RT
Proteinase K	1.4ml	7ml	28ml	2-8 °C
Hand Book	1	1	1	- -
Certificate of Quality	1	1	1	- -

Note:

1. Proteinase K is provided in the kit. 1.25ml of Proteinase K (20mg/ml or 600mAU/ml) is required for 50 spin columns.

9. Specifications

Weight of stool sample	**180-220mg**
Capacity of column reservoir	700µl
Binding capacity of membrane (ds DNA)	100µg
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 180-220mg.

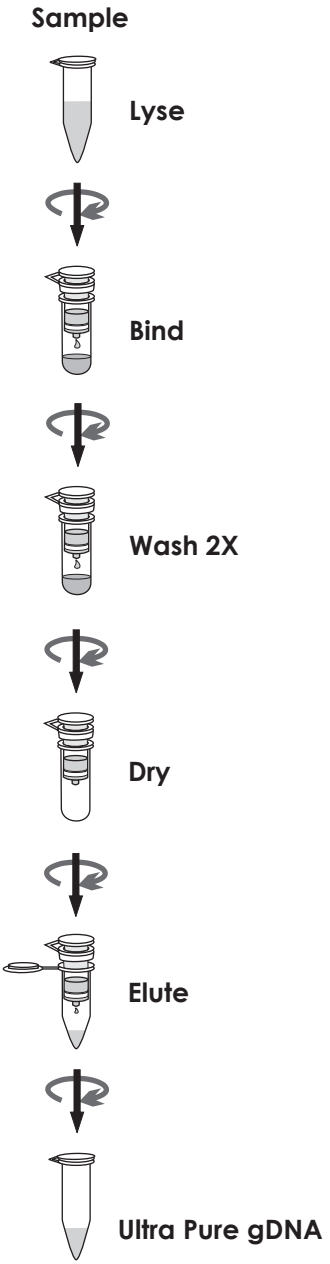
Don'ts

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

C. 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 'SE'.

11. mdi Stool gDNA Miniprep Procedure



12. Stool gDNA Isolation

12.1 Principle

Obtaining highly pure gDNA from stool using **mdi** Stool gDNA Miniprep Kit involves:

Lysis of Stool Sample

For efficient lysis mix stool sample completely with Proteinase K and buffer SB, 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 SB.

Washing

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

Washing is done by buffer SW1 and SW2.

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 'SE'.

12.2 Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Maximum amount of stool sample should be 180 - 220 mg.

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 stool sample completely with Proteinase K and buffer SB, then incubate at 56°C.
2. Proteinase K should be added to sample before addition of buffer SB to inactivate nuclease that can degrade DNA during purification.
3. In case of any precipitation in buffer SB, re-dissolve by warming to 56°C for few minutes.

Washing

1. To remove residual wash buffer, spin the column with closed lid for 1 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, repeat elution step.

Yield

DNA yield is typically 15-60 µg, but depending on the individual stool sample and the way it was stored may range from 5 - 100 µg. Typically DNA concentration is 75 - 300 ng/µl.

12.3 Protocol (Isolation of DNA from Human/Animal Stool for Pathogen Detection)

Note: Things to do before starting

1. Heat a water bath at 70°C and 56°C
2. Arrange ethanol (96-100%)
3. Arrange 2ml and 1.5ml microcentrifuge tube

Procedure:

1. Weigh 180-220mg stool in a 2ml microcentrifuge tube (not provided) and place the tube on ice.

   Note: When using frozen stool samples, do not allow the sample to thaw before addition of Buffer MSL, otherwise the DNA in the sample may degrade. All steps should be performed at room temperature after adding Buffer MSL.
2. Add 1.2ml Buffer MSL to each stool sample. Vortex Continuously for 5 minutes or until the stool sample is thoroughly homogenized.
3. Incubate the sample at 70°C for 5 minutes. This step increases total DNA yield 3 to 5 fold and helps to lyse bacteria and other parasites. The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).
4. Vortex for 15 seconds and centrifuge at 10,000 rpm for 5 minutes to pellet stool particles.
5. Pipet 15µl Proteinase K into a new 1.5ml microcentrifuge tube (not provided).
6. Add 200µl supernatant from step - 4.
7. Add 200µl Buffer SB to the sample and mix thoroughly by vortexing.

   Note: Do not add Proteinase K directly to Buffer SB.
8. Incubate at 56°C in water bath for 15 minutes. Mix 2-3 times by inverting the tube during incubation.

9. Briefly centrifuge the sample after incubation to remove drops from inside of lid.
10. Add 200µl Ethanol, mix well by vortexing and briefly centrifuge the sample to remove drops from inside of lid.
11. Place the spin column in the collection tube, pass the above sample through the spin column by spinning at ≥ 10,000 rpm for 1 minute. Discard collection tube containing flowthrough.

    Note: Maximum volume of the column reservoir is 700µl. For sample volumes >700µl, simply load remaining sample balance and spin at ≥ 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.
12. Place the spin column in the new collection tube. Wash the column with 500µl of buffer SW1 by centrifuging for 1 minute at ≥ 10,000rpm. Discard collection tube containing flowthrough.
13. Place the spin column in the new collection tube. Wash the column with 500µl of buffer SW2 by centrifuging for 3 minute at ≥ 10,000rpm. Discard collection tube containing flowthrough.
14. Place the spin column in the new collection tube with closed lid and centrifuge at ≥ 10,000rpm for 1 minute to completely remove the wash buffer.
15. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided)

    Elute the bound gDNA by adding 200µl of buffer SE directly to the center of the column membrane and let it stand for 2 minutes. Spin at ≥ 10,000rpm for 1 minute.
16. **Optional:** For higher concentration, 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 100µl of buffer SE.

12.4 Protocol (Isolation of DNA from Human/Animal Stool for Pathogen Detection using Stool Tubes)

For easier measurement of stool samples, stool tubes with integrated measuring spoons in their lids are available. The spoons in these tubes are designed to collect 200 mg stool.

Note: Things to do before starting

1. Heat a water bath at 70°C and 56°C
2. Arrange ethanol (96-100%)
3. Arrange 2ml and 1.5ml microcentrifuge tube

Procedure:

1. Use the spoon integrated into the cap of a stool tube (not provided) to measure 180-220 mg of the stool sample, A level spoonful will correspond to approximately 200 mg stool. Close the tube and place it on ice.

   A clean spatula should be used to remove excess stool from spoon.
2. Add 1.2ml Buffer MSL to each stool sample. Vortex continuously for 5 minutes or until the stool sample is thoroughly homogenized.
3. Transfer the lysate into 2 ml microcentrifuge tube and continue the procedure from step-3 of protocol (Isolation of DNA from stool for pathogen detection) page number 10.

12.5 Protocol (Isolation of DNA from Stool for Human/Animal DNA analysis)

Lysis conditions in this protocol are optimized to increase the yield of Human or Animal Genomic DNA

Note: Things to do before starting

1. Heat a water bath at 56°C
2. Arrange ethanol (96-100%)
3. Arrange 2ml and 1.5ml microcentrifuge tube

Procedure:

1. Weigh **180-220mg** stool in a 2ml microcentrifuge tube (not provided) and place the tube on ice.

   Note: When using frozen stool samples, do not allow the sample to thaw before addition of Buffer MSL, otherwise the DNA in the sample may degrade. All steps should be performed at room temperature after adding Buffer MSL.
2. Add 1.6ml Buffer MSL to each stool sample. Vortex Continuously for 5 minutes or until the stool sample is thoroughly homogenized.
3. Centrifuge sample at ≥10,000 rpm for 5 minutes.
4. Pipet 25µl Proteinase K into a new 2ml microcentrifuge tube (not provided).
5. Add 600µl supernatant from step - 3.
6. Add 600µl Buffer SB to the sample and mix thoroughly by vortexing.

   Note: Do not add Proteinase K directly to Buffer SB.
7. Incubate at 56°C in water bath for 15 minutes. Mix 2-3 times by inverting the tube during incubation.
8. Briefly centrifuge the sample after incubation to remove drops from inside of lid.

9. Add 600µl Ethanol, mix well by vortexing and briefly centrifuge the sample to remove drops from inside of lid.
10. Place the spin column in the collection tube, pass the above sample through the spin column by spinning at ≥ 10,000 rpm for 1 minute. Discard collection tube containing flowthrough.

    Note: Maximum volume of the column reservoir is 700µl. For sample volumes >700µl, simply load remaining sample balance and spin at ≥ 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.
11. Place the spin column in the new collection tube. Wash the column with 500µl of buffer SW1 by centrifuging for 1 minute at ≥ 10,000rpm. Discard collection tube containing flowthrough.
12. Place the spin column in the new collection tube. Wash the column with 500µl of buffer SW2 by centrifuging for 3 minute at ≥ 10,000rpm. Discard collection tube containing flowthrough.
13. Place the spin column in the new collection tube with closed lid and centrifuge at ≥ 10,000rpm for 1 minute to completely remove the wash buffer.
14. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided)

    Elute the bound gDNA by adding 200µl of buffer SE directly to the center of the column membrane and let it stand for 2 minutes. Spin at ≥ 10,000rpm for 1 minute.
15. **Optional:** For higher concentration, 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 100µl of buffer SE.

12.6 Protocol (Isolation of DNA from Stool for Human/Animal DNA analysis using Stool Tubes)

For easier measurement of samples, stool tubes with integrated measuring spoons in their lids are available. The spoons in these tubes are designed to collect 200 mg stool.

Note: Things to do before starting

1. Heat a water bath at 70°C and 56°C
2. Arrange ethanol (96-100%)
3. Arrange 2ml and 1.5ml microcentrifuge tube

Procedure:

1. Use the spoon integrated into the cap of a stool tube (not provided) to measure **180-220 mg** of the stool sample, A level spoonful will correspond to approximately 200 mg stool, close the tube and place it on ice.

   A clean spatula should be used to remove excess stool from spoon.
2. Add 1.6ml buffer MSL to each stool sample. Vortex Continuously for 5 minutes or until the stool sample is thoroughly homogenized.
3. Transfer the lysate into 2 ml microcentrifuge tube and continue the procedure from step-3 of protocol (Isolation of DNA from stool for Human/Animal DNA analysis)page number 13.

12.7 Protocol (Isolation of DNA from Larger Volumes of Stool)

This protocol is recommended when the target DNA is not distributed homogeneously in the stool. Using a relatively larger amount of starting material enhances the chances of isolating DNA from low titer sources in the stool samples.

Procedure:

1. Weigh the stool sample and add 10 volumes of buffer MSL. Vortex vigorously for 5 minutes or until the sample is completely homogenized.

   For example, add 10 ml buffer to 1 gram stool sample.

2. Continue any of the following protocols by pipetting recommended volume of lysate.

   A. Isolation of DNA from stool for pathogen detection

   Use 1.2 ml of lysate from step-1 and continue from step-3 at page 10.

   B. Isolation of DNA from stool for Human/Animal DNA analysis

   Use 1.6 ml of lysate from step-1 and continue from step-3 at page 13.

   NOTE: To make the pipetting easy for viscous sample (lysate) cut the end of pipette tip.

13. Trouble Shooting Guide

A. Little or no Yield of gDNA

1. Overloading of spin column	Do not exceed the starting material
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 SB and ethanol before loading on to the spin column	Add buffer SB 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 t o -70°C. Avoid repeated freezing and thawing of sample.
9. Improper storage of stool samples	For short term storage, stool samples should be stored at 4°C. For long term, store at -20°C to -70°C.

B: Low quality DNA

1. Nuclease contamination	Use autoclaved plastic and glassware.
2. Sheared Genomic DNA	Avoid vigorous mixing.

C: DNA does not perform well

Residual wash buffer in eluate	Spin the column with closed lid for 1-2 minutes at $\geq$10,000 rpm after discarding flowthrough.

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

DNA diluted with water instead of buffer	Use buffer SE 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 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
STGK	STGK					50	0050
						250	0250
						1000	1000

Example:

STGK	XX	XX	XX	P	0250

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