

Micro Gel Extraction Kit



User Guide

mdi
Membrane Technologies

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1. Introduction

mdi Micro Gel Extraction Kit is a quick, convenient and economical tool to obtain highly concentrated DNA fragments (70bp-4kb) after gel analysis from standard or low melt Agarose gel. The buffer system provided in the kit facilitates efficient binding of DNA fragments from solubilised gel slice onto the spin column.

Washing is done with help of wash buffers in order to remove agarose gel, salts, and other impurities from DNA samples. Purified DNA is eluted in small volumes (10µl) of low salt buffer or water for variety of downstream applications.

2. Applications

1. Automated Fluorescent Sequencing
2. Radioactive Sequencing
3. Restriction Digestion
4. Ligation
5. Transformation
6. Cloning
7. PCR

3. Storage Conditions

As soon as you receive the kit, open it and store **mdi** Micro Gel Extraction spin columns at 2-8°C.

The remaining kit components can be stored at room temperature. The kit is stable for 1 year at given storage conditions. If precipitates appear in buffers re-dissolve at 37°C for few minutes.

4. Quality Assurance

The mdi Micro Gel Extraction 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 Micro Gel Extraction kit.

6. Lot Release Criteria

Each lot of Micro Gel Extraction 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 system 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	2 - 8°C
Collection Tubes	50	250	1000	RT
Buffer MMG	100ml	500ml	2000ml	RT
Buffer GW	60	300	1200	RT
Buffer GE	15	75	300	RT
Hand Book	1	1	1	-
Certificate of Quality	1	1	1	-

9. Specifications

Capacity of column reservoir	800µl
Binding capacity of membrane (ds DNA)	5µg
Recovery of DNA (70bp-4kb)	80%
Maximum weight of gel slice	400mg
Minimum elution volume for conc. DNA	10µl
Total eluate volume	9µl

10. Principle

The **mdi** Micro Gel Extraction kit allows isolation of high ultra pure DNA fragments from solubilised agarose gel which involves:

1. Capturing of DNA from solubilised gel slice on spin column
2. Washing
3. Elution

1. Capturing of DNA from solubilised gel slice on spin column

In order to facilitate adsorption of DNA fragment on to the spin column, suitable pH and salt concentration are required which is achieved by addition of binding buffer 'MMG'.

2. Washing

Subsequent to DNA binding from solubilised gel slice on spin column unwanted components like dyes, salts and agarose are washed away. Washing is done by buffer 'GW'.

3. Elution

Salt concentration and pH of elution buffer is important for elution efficiency, elution occurs at basic conditions and low salt concentration in small volumes. Elution is done with buffer 'GE'.

11. Important Points to be Considered

Optimization of Binding Buffers

The binding buffers should possess appropriate salt concentration and pH to facilitate the efficient binding of single or double stranded DNA (70bp - 4kb) from solubilized gel slice on spin column.

Centrifugation

- * All centrifugation steps should be carried out at room temperature at $\geq 10,000$ rpm in a conventional table top microcentrifuge.

In case of choking of spin column, increase centrifugation time and centrifugation speed to 12000rpm. Check the dissolution of the gel before addition to the column to avoid choking of the spin column

Washing

- * To remove residual wash buffer spin the column for 1-2 minutes at $\geq 10,000$ rpm.
- * Flow through should be properly discarded before additional centrifugation step otherwise it will not be removed & may inhibit enzymatic reactions.

Elution

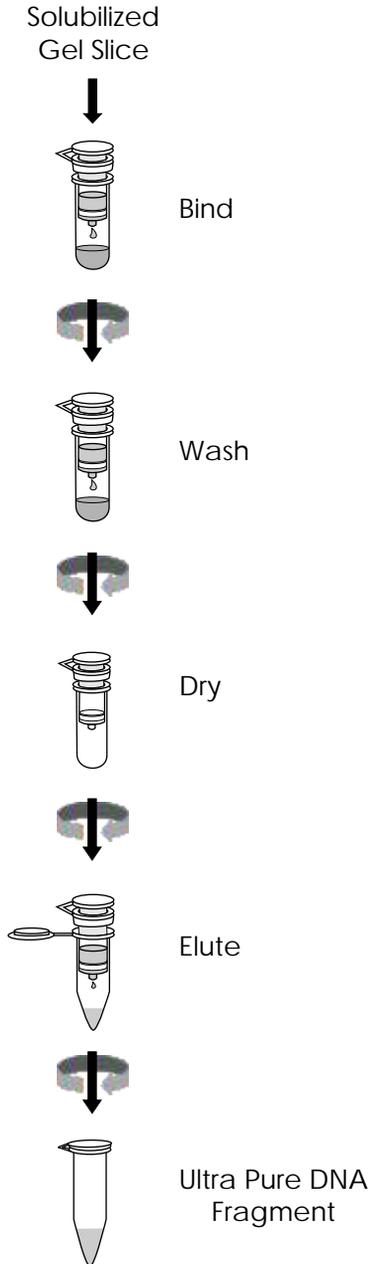
- * Elution buffer should be dispensed on to the center of column for maximum elution efficiency. If eluate volume is lower, then centrifuge for one minute extra at $\geq 10,000$ rpm.

Yield

DNA yield depends on following factors:

1. Volume of elution buffer
2. Amount of starting material

12. mdi Micro Gel Extraction Procedure



13. Protocol

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel or blade. (Minimize the size of the gel slice by removing extra agarose).
2. Weigh the gel slice in a colorless tube. Add 3 volumes of buffer MMG to 1 volume of gel. (100mg ~ 100 μ l) For example, add 300 μ l of buffer MMG to each 100mg of gel.
3. Incubate at 50 °C in a water bath for 10 minutes (or until the gel slice has completely dissolved). To help dissolve the gel, mix by vortexing the tube every 2-3 minutes, during incubation.
4. After the gel slice has dissolved completely, add 1 gel volume of Isopropanol to the sample and mix by inverting the tube. For example, if the agarose gel weight is 100 mg, add 100 μ l isopropanol.
5. Place the spin column in the collection tube in a suitable rack. To bind DNA, load the sample to the spin column and centrifuge for 1 minute at $\geq 10,000$ rpm.

The maximum volume of the column reservoir is 800 μ l. For sample volumes of more than 800 μ l, simply load, balance micro centrifuge and spin again.

6. Discard the flowthrough, and using the same collection tube, wash the spin column with 500 μ l of buffer MMG by centrifuging for 1 minute at $\geq 10,000$ rpm.
7. Discard the flowthrough, and using the same collection tube, wash the spin column with 750 μ l of buffer GW by centrifuging for 1 minute at $\geq 10,000$ rpm.
8. Discard the flowthrough, place the spin column in the same collection tube and centrifuge for 1-2 minutes at $\geq 10,000$ 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.

9. Place the spin column in a fresh 1.5ml microfuge tube (Not provided). Elute the DNA by dispensing 10 μ l of elution buffer GE directly on to the center of the column membrane, allow it to stand for 1 minute and centrifuge for 1 minute at $\geq 10,000$ rpm.

Note: The elution buffer should be dispensed on to the center of the column membrane for maximum recovery. The average eluate volume is 9 μ l from 10 μ l elution buffer. If the eluate volume is lower, then centrifuge for one minute extra at $\geq 10,000$ rpm.

14. Trouble Shooting Guide

A. Little or no Yield of DNA

1. Gel slice not solubilized

Mix thoroughly with buffer MMG by completely vortexing the tube every 2-3 minutes during 50 °C incubation.

Increase incubation time to 15 minutes.

Preferably incubation should be done in a water bath instead of an oven.

2. Gel slice >400 mg

Use multiple columns for gel slices > 400 mg.

3. pH of electrophoresis buffer too high

Check the pH of electrophoresis buffer before use. In case it is > 8, prepare fresh buffer.

The electrophoresis buffer, if incorrectly prepared or used repeatedly, results in sample pH that exceeds the buffering capacity of buffer MMG and leads to inefficient DNA binding.

4. Improper dispensing of elution buffer

Elution buffer must be dispensed on to the center of the column membrane for maximum elution efficiency.

5. Insufficient incubation of elution buffer in the spin column

Increase incubation time 2-3 minutes more.

B. DNA does not perform well

1. Eluate contaminated with Agarose

The gel slice is not properly solubilized or is more than 400mg in weight. Vortex every 2-3 minutes during incubation time. Increase total incubation time.

2. Elution contains residual wash buffer

Spin the column for 1-2 minutes extra at $\geq 10,000$ rpm to remove the residual wash buffer completely.

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 quality 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	X	Pack Size	
Type	Code					Pack Size	Code
MGEK	MGEK					50	0050
						250	0250
						1000	1000

Example:

MGEK	XX	XX	XX	X	0250
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