Gel Extraction Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

mdi Gel Extraction Kit is a quick, convenient and economical tool to isolate DNA fragments (70bp-10kb) after gel analysis from standard or low melt Agarose gel. The buffer system provided in the kit facilitates efficient binding of DNA fragments from solubilized gel slice on spin column.

Washing is done with the help of wash buffers in order to remove agarose gel, salts, and other impurities from DNA samples. Purified DNA is eluted in small volumes 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
4. Cloning
5. PCR

3. Storage Conditions

mdi Gel Extraction Kit should be stored at room temperature. The kit is stable for one year at above storage conditions without showing any reduction in performance and quality.

For longer storage, the entire kit can be stored at 2-8°C. In case precipitates are observed in buffer, re-dissolve all buffers before use at 37°C for few minutes. All buffers should be at room temperature before starting the protocol.

4. Quality Assurance

The mdi 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 Gel Extraction Kit.

6. Lot Release Criteria

Each lot of 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	RT
Collection Tubes	50	250	1000	RT
Buffer MG	100ml	500ml	2000ml	RT
Buffer GW	100ml	500ml	2000ml	RT
Buffer GE	15ml	75ml	300ml	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)	10µg
Recovery of DNA (70bp-10kb)	70-80%
Maximum weight of gel slice	400mg
Minimum elution volume	30µl
Total eluate volume	28µl

10. Principle

Obtaining highly pure DNA fragments from solublised agarose gel using mdi Gel Extraction Kit involves: Capturing of DNA from solublised gel slice on spin column, Washing and Elution.

1. Capturing of DNA from solublised 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 'MG'.

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'.

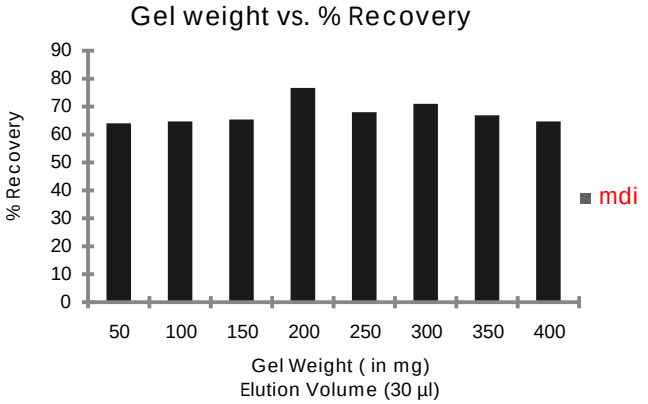
3. Elution

   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'.

4. Yield and Concentration

   DNA Yield depends on following factors:

   1. Gel weight
   2. Volume of elution Buffer
   3. Point of application of elution buffer on the column
   4. Incubation period of elution buffer on the column



Test Conditions

Fragment Size: 9 kb

DNA Feed: 14.2µg

Elution Volume: 30µl

5. Yield analysis through Agarose Gel Electrophoresis

DNA Yields can be determined using Agarose Gel Electrophoresis. Total yield and recovery obtained from 1µg and 0.5µg starting DNA are illustrated in table given below

Starting DNA	Recovery	Total yield (30 µl eluate)	Amount of DNA in 1µl
1µg	95%	950ng	31ng
	80%	800ng	26ng
0.5µg	95%	480ng	16ng
	80%	400ng	13ng

6. Quantification of DNA fragments

DNA fragments can be quantified by running a sample simultaneously with a known amount of same sized DNA fragments.

A visual comparison of the band intensity of both loaded DNA sample and standard will help quantify the loaded DNA (known DNA quantity)

11. Important Points to be Considered

Optimization of Binding Buffers

The binding buffers should posses appropriate salt concentration and pH to facilitate the efficient binding of single or double stranded DNA (70bp-10kb) from solublised gel slice on spin column.

Centrifugation

All centrifugation steps should be carried out at room temperature at ≥10,000 rpm in a conventional table top microcentrifuge.

In case of choking of spin column, increase centrifugation time.

Increase centrifugation speed to ≥12000 rpm. Check the dissolution of the gel before addition to the column to avoid chocking of the spin column.

Washing

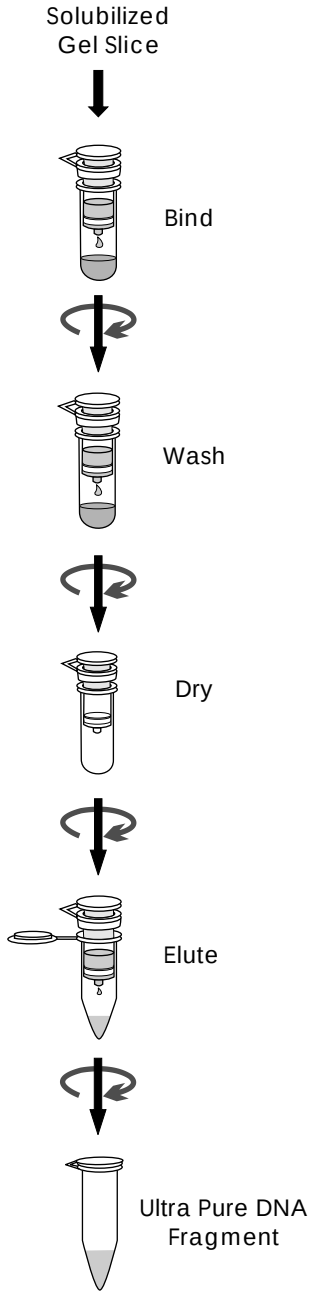
To remove residual wash buffer spin the column for 1-2 minutes at ≥10,000 rpm.

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 ≥10,000 rpm.

12. mdi 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 MG to1 volume of gel. (100mg ~ 100µl). For example, add 300µl of buffer MG to each 100mg of gel.

3. Incubate at 50 °C in a water bath for 10 minutes (or untill 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 100µl 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 ≥10,000 rpm.

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

6. Discard the flowthrough, and using the same collection tube, wash the spin column with 500µl of buffer MG by centrifuging for 1 minute at ≥10,000 rpm.

7. Discard the flowthrough, and using the same collection tube, wash the spin column with 750µl of buffer GW by centrifuging for 1 minute at ≥10,000 rpm.

8. Discard the flowthrough, place the spin column in the same collection tube and centrifuge for 1-2 minutes 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.

9. Place the spin column in a fresh 1.5ml microfuge tube (not provided). Elute the DNA by dispensing 30µl 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 ≥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 28µl from 30µl elution buffer. If the eluate volume is lower, then centrifuge for one minute extra at ≥10,000 rpm.

14. Trouble Shooting Guide

A. Low or no recovery

1. Gel slice not solubilized completely	Mix thoroughly with buffer MG by 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 a sample pH that exceeds the buffering capacity of buffer MG 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 400 mg 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 diagnostic 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	X	Pack Size	
Type	Code					Pack Size	Code
SGEK	SGEK					50	0050
						250	0250
						1000	1000

Example:

SGEK	XX	XX	XX	X	0250

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