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BIO BASIC INC.

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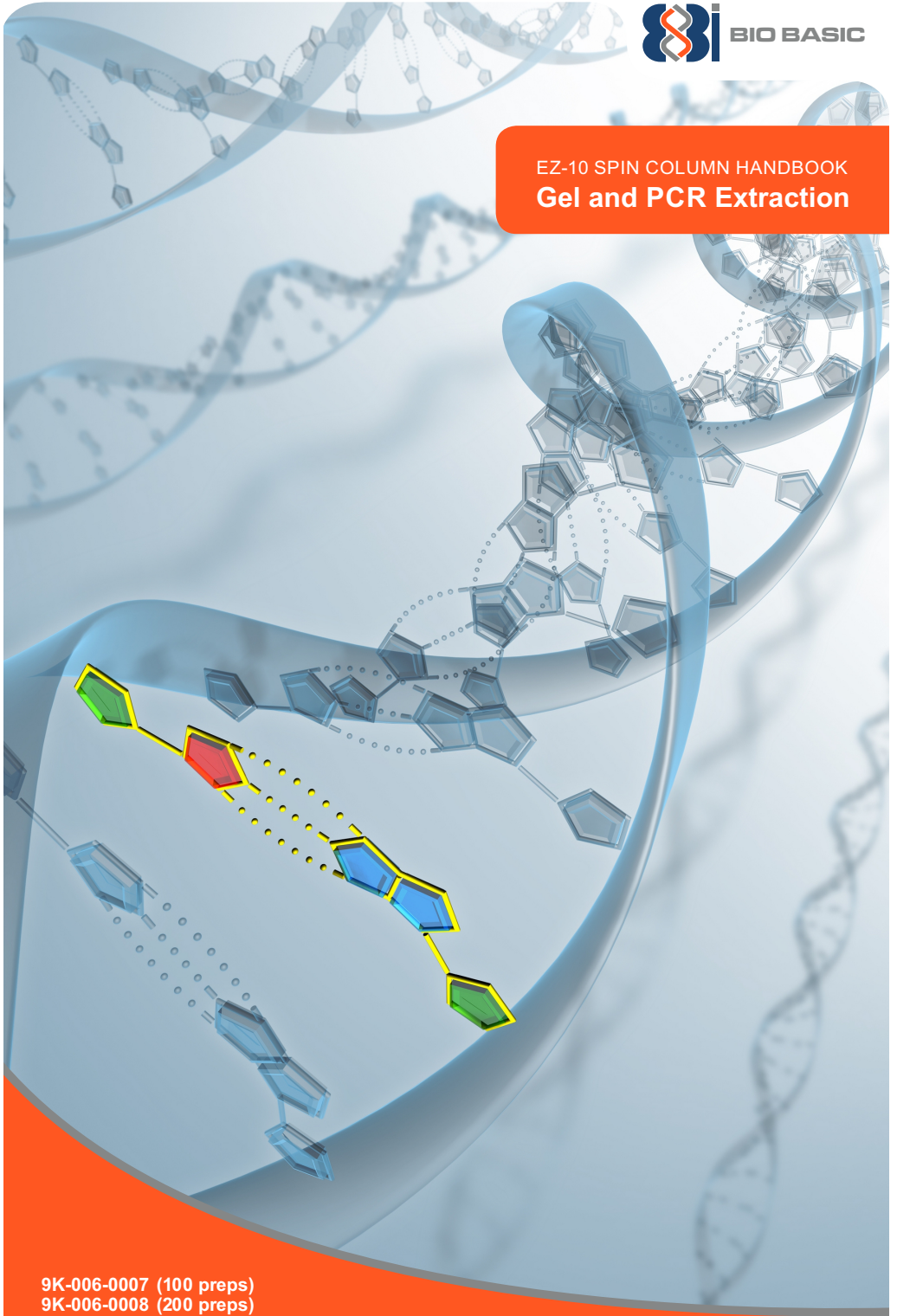
EZ-10 SPIN COLUMN HANDBOOK

Gel and PCR Extraction



9K-006-0007 (100 preps)
9K-006-0008 (200 preps)

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For Research Use Only

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2) Samples floats upon loading in agarose gel
The sample contains ethanol from washing step. Discard the liquid waste from the collection tube after washing step, and spin again for additional two minutes before the final elution step.

3) For optimal results in downstream DNA sequencing, an additional washing step is recommended.

Other Kits Available

EZ-10 Spin Column Plasmid DNA MiniPreps Kit
BS413 (50preps), BS414 (100preps), BS614 (250preps)

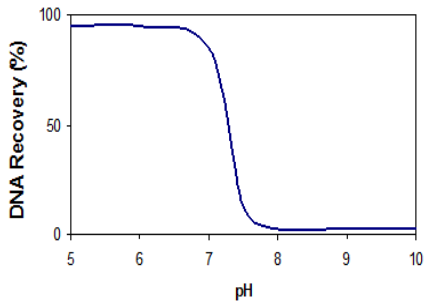
EZ-500 Spin Column Plasmid DNA MaxiPreps Kit
BS4654 (4preps), BS466 (20preps)

EZ-10 96-Well Spin Column PCR Products Purification Kit
BS3652 (2preps), BS365 (5preps)

And much more…

piece containing DNA will have a high pH environment. As a result, DNA will bind poorly to the silica membrane. This can be illustrated in table 1.1.

Table1.1: DNA Recovery (%) Against pH



Troubleshooting Guide: EZ-10 Spin Column PCR Products Purification Kit and DNA Gel Extraction Kit

1) Low Yield
   There are number of variables that can cause low yield
   a. Each step has to be strictly followed.
   b. Make sure column binding capacity 10µg is not exceeded.

Introduction

The Gel and PCR Extraction System provides a simple and efficient method for extraction of DNA from agarose gels, and purification of DNA from enzymatic reactions such as PCR or restriction enzyme digestions.

The DNA is selectively adsorbed in silica gel-based EZ-10 column and other components are washed away. The DNA is then eluted off the column and can be used for any downstream applications.

The purification method used in these protocols does not require use of phenol, chloroform, or CsCl. The DNA is purified without an additional step of ethanol precipitation.

Limitations of Use

These kits are designed for research use only. It is also not to be used for human diagnostic or drug production purposes.

Features

- √ Simple, Fast and Efficient
- √ Preparation of high quality DNA which can be used in any downstream applications such as sequencing, PCR, transformation or restriction digestions
- √ High Yield and Reproducible
- √ High Capacity - Up to 10µg of DNA per column

Applications

This kit can be used for purification of DNA from 40bp-40kb

Storage

Gel and PCR Extraction System should be stored dry at room temperature (15°C-25°C). Kits can be stored for up to 24 months without showing any reduction in performance and quality.

Quality Control

Each lot of EZ-10 Spin Column kit is tested against predetermined specifications to ensure consistent product quality.

3. Add **750µl** of Wash Solution, and centrifuge at 10,000rmp for 1 minute. Discard the solution in the tube.
4. Repeat step 3. Spin at 10,000rpm for an additional minute to remove any residual Wash Buffer.
5. Place the column in a clean 1.5ml microfuge tube. Add **30-50µl** of Elution Buffer to the center of the column and incubate at room temperature for 2 minutes.

Note: It is extremely important to add the Elution Buffer into the center part of the column. Incubating the column with the Elution Buffer at higher temperature (37ºC to 50ºC) may slightly increase the yield especially for large (>10,000bp) DNA Plasmids. Prewarming the Elution Buffer at 55ºC to 80ºC may also slightly increase elution efficiency.

6. Centrifuge at 10,000rpm for 2 minutes to elute the DNA.
7. Store purified DNA at -20ºC.

NEW BINDING BUFFER II WITH PH INDICATOR

Adsorption of DNA to silica membrane depends on pH, it is typically 95% if the pH is <7.0. At a higher pH binding is drastically reduced. When the electrophoresis buffer has been used repeatedly, incorrectly prepared, or used in an enzymatic reaction and is strongly basic, the agarose gel

for 2 minutes and discard the flow-through in the tube.

4. Add **750µl** of Wash Solution, and centrifuge at 10,000rpm for one minute. Discard the solution in the tube.
5. Repeat step 4. Centrifuge at 10,000rpm for an additional minute to remove any residual Wash Buffer.
6. Place the column in a clean 1.5ml microfuge tube. Add **30-50µl** of Elution Buffer to the center of the column and incubate at room temperature for 2 minutes. Centrifuge at 10,000rpm for 2 minutes to elute DNA.

Note: It is extremely important to add the Elution Buffer to the center of the column. Incubating the column at higher temperature (37ºC to 50ºC) may slightly increase the yield. Pre-warming the Elution Buffer at 55ºC to 80ºC may also slightly increase elution efficiency.

7. Store purified DNA at -20ºC.

Protocol for DNA purification from enzymatic reactions

1. Transfer entire contents of the reaction mixture to a 1.5ml microfuge tube and add **3 volumes** of Binding Buffer II. Mix by inverting the tube a few times.
2. Add the above mixture to the EZ-10 column and let the column stand for 2 minutes. Centrifuge at 10,000rpm for 1 minute and discard the flow-through in the tube.

Components:

Gel and PCR Extraction System	9K-006-0007 100 Preps	9K-006-0008 200 Preps
Binding Buffer II	2X50ml	4X50ml
Wash Solution(A)	2X20ml	2X40ml
Elution Buffer	10ml	20ml
EZ-10 Columns	100	200
Protocol	1	1

(A) Before use, add 160ml of 96-100% ethanol to 40ml Wash Solution for 9K-006-0007; add 2X160ml of 96-100% ethanol to 2X40ml Wash Solution for 9K-006-008. For other volumes of Wash Solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol: volume of Wash Solution = 4:1).

(B) Elution Buffer is 2mM Tris-HCl pH 8.0~8.5. Although TE buffer pH 8.0 or water may be substituted, the resulting yields may be up to 20% lower.

Principle:

EZ-10 spin column purification kits utilize a silica-gel membrane that selectively absorbs up to 10µg of DNA fragments in the presence of specialized binding buffers. Nucleotides, oligos (<40-mer), enzymes, mineral oil and other impurities do not bind to the membrane and are washed away. The DNA fragments can then be eluted off the column

in small volume and used in downstream applications without further processing.

Protocol for Purification of PCR Products

1. Transfer PCR reaction mixture to a 1.5ml microfuge tube and add 3 volumes of **Binding Buffer II**.
2. Transfer the above mixture solution to the EZ-10 column and let it stand at room temperature for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes.
3. Remove the flow-through in the tube. Add **750µl** of Wash Solution to the column and centrifuge at 10,000 rpm for 2 minutes.
4. Repeat washing procedure in step 3. Spin at 10,000 rpm for an additional minute to remove any residual Wash Solution.
5. Transfer the column into a clean 1.5ml microfuge tube and add **30-50µl** of Elution Buffer. Incubate at room temperature for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes to elute the DNA.

Note: It is extremely important to add the Elution Buffer into the center part of the column. Incubating the column with the Elution Buffer at higher temperature (37ºC to 50ºC) may slightly increase the yield especially of large (>10,000 bp) DNA plasmids. Prewarming the Elution Buffer at 55ºC to 80ºC may also slightly increase elution efficiency.

6. Store purified DNA at -20ºC.

Note:

1. If PCR reaction mixture contains seriously non-specific amplified DNA fragments, use of the DNA Gel Extraction Kit is recommended.
2. This kit can not remove the template and primers with chain length longer than 40-mer.

Protocol for Agarose Gel

1. Excise the DNA fragment from the gel with a clean, sharp scalpel. Weigh the gel slice and transfer to a 1.5mL microfuge tube.
2. Add **400µl of Binding Buffer II** for each 100mg of gel weight (for example, a gel slice weighing 125mg would require 500µl of Binding Buffer II). Incubate at 50^0C-60^0C for 10 minutes and shake occasionally until agarose is completely dissolved. For high concentration gels (1.5-2.0%), 700µl of Binding Buffer II per 100mg of agarose gel are added.

Note: After addition of binding buffer, carefully monitor the color of the binding mixture. If the binding mixture is **yellow**, then optimal pH has been obtained; continue with the rest of extraction steps. However, if the binding mixture turns a blue or purple color, adjust pH by adding a small volume of 3 M sodium acetate (pH 5.0) until optimal pH is reached. Precede with the rest of the extraction steps.

3. Add the above mixture to the EZ-10 column and let stand for 2 minutes. Centrifuge at 10,000rpm