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BIO BASIC INC.

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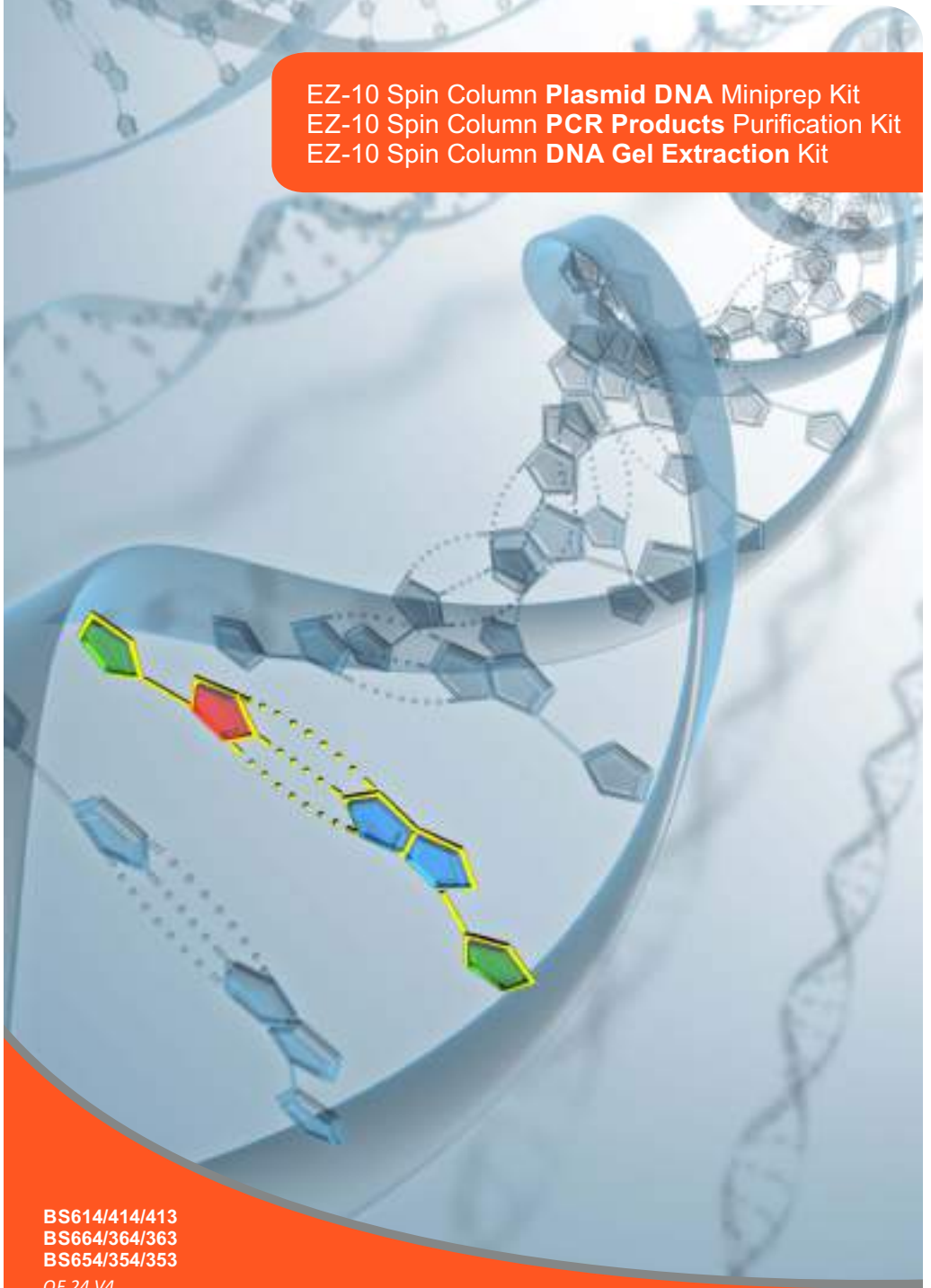
EZ-10 Spin Column **Plasmid DNA** Miniprep Kit
EZ-10 Spin Column **PCR Products** Purification Kit
EZ-10 Spin Column **DNA Gel Extraction** Kit



BS614/414/413
BS664/364/363
BS654/354/353

QF 24 V4
CV1 2019

For Research Use Only

Table of Contents

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-200 Spin Column Plasmid DNA MediPreps Kit
BS4634 (4preps), BS464 (20preps)

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

EZ-10 Spin Column Plasmid DNA MiniPreps Kit
BS413, BS414

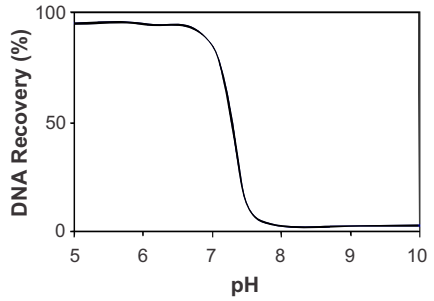
EZ-10 Spin Column PCR Products Purification Kit
BS363, BS364

EZ-10 Spin Column DNA Gel Extraction Kit
BS353, BS354

PRODUCTS ARE FOR SCIENTIFIC RESEARCH ONLY
NOT INTENDED FOR HUMAN OR ANIMAL USE

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.

Table 1.1: DNA Recovery (%) Against pH



Troubleshooting Guide: E-Z 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 EZ-10 Spin Column Kits provide a simple and efficient method for purification of plasmid DNA, 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. The purified plasmid DNA should not be used for live animal transfections. 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

EZ-10 Spin Column Plasmid DNA Minipreps Kit BS413, BS414, BS614

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

EZ-10 Spin Column PCR Products Purification Kit BS363, BS364, BS664

Recovery of 40bp-40kb DNA fragments from reaction solutions

EZ-10 Spin Column DNA Gel Extraction Kit BS353, BS354, BS654

Recovery of 40bp-40kb DNA fragments from agarose gels

Storage

EZ-10 Spin Column Kits 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. RNase A stock solution can be stored for 2 years at 4°C. After addition of RNase A, Solution I is stable for 6 months at 4°C.

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 silicamembrane 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 repeatedl 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.

Protocol: EZ-10 Spin Column Plasmid DNA Minipreps Kit BS413, BS414, BS614

EZ-10 Spin Column Plasmid DNA Minipreps Kit Components	**BS413 50 Preps**	**BS414 100 Preps**	**BS614 250 Preps**
RNase A Solution (10mg/ml)	120µl	240µl	600µl
Solution I	6ml	12ml	30ml
Solution II	12ml	24ml	2X30ml
Solution III	25ml	2X25ml	5X25ml
VisualLyse	60µl	120µl	300µl
Wash Solution	20ml	2X20ml	2X40ml
Elution Buffer	5ml	10ml	25ml
EZ-10 Column	50	100	250
Protocol	1	1	1

a) Before use, add the RNase A Solution to the bottle containing Solution I and mix well. Solution I with RNase A should be stored at 4ºC for frequent use and at -20ºC for infrequent use.
b) Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37ºC.
c) Before use, add 80ml of 96-100% of ethanol to 20ml Wash Solution for BS413; add 160ml of 96-100% ethanol to 40ml Wash Solution for BS414; add 320ml of 96-100% ethanol to 80ml Wash Solution for BS614. 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).

d) Elution Buffer is 2.0 mM 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:

This kit provides a simple and efficient method for mini plasmid DNA purification. The plasmid DNA is selectively adsorbed in silica gel-based EZ-10 column and other impurities such as proteins, salts, nucleotides, oligos (<40-mer) are washed away. In order to maximize the recovery yield of plasmid DNA, a color indicator-VisualLyse is added to the buffer which prevents insufficient or over-lysis during lysis and neutralization step. The plasmid DNA is then eluted off the column and can be used for any downstream application.

Protocol for Purification of Plasmid DNA

1. Add **1.5 - 5mL** overnight culture in the tube and centrifuge at 12,000rpm for 2 minutes. Drain the liquid completely. For low copy number plasmid, see the protocol on the following page.
2. Add **100µl of Solution I** to the pellet, mix well, and keep for 1 minute.
3. Add **1µl of VisualLyse** to the mixture above.

Note: addition of VisualLyse is an optional step.

4. Add **200µl of Solution II** to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute. To prevent contamination from genomic DNA, do not vortex. If VisualLyse has been added, 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. DNA fragments are then eluted off the column and can be used for downstream applications without further processing.

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

Protocol: EZ-10 Spin Column DNA Gel Extraction Kit BS353, BS354, BS654

EZ-10 Spin Column DNA Gel Extraction Kit Components	BS353 50 Preps	BS354 100 Preps	BS654 250 Preps
Binding Buffer II	50ml	2X50ml	5X50ml
Wash Solution	20ml	2X20ml	2X40ml
Elution Buffer	5ml	10ml	25ml
EZ-10 Column	50	100	250
Protocol	1	1	1

(A) Before use, add 80 ml of 96-100% of ethanol to 20ml Wash Solution for BS353; add 160ml of 96-100% ethanol to 40ml Wash Solution for BS354; add 320ml of 96-100% ethanol to 80ml Wash Solution for BS654. 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.

Note: The kit is observed to have better performance when TAE, rather than TBE, is used.

Principle:

The EZ-10 spin column purification kit utilizes a silica-gel based membrane which selectively adsorbs up to 10µg of DNA fragments in the

solution will turn blue after addition of Solution II. A homogenously blue suspension should also be observed. If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.

5. Add **350µl of Solution III**, and mix gently. Incubate at room temperature for 1 minute. A fluffy white material forms and lysate should become less viscous. If VisualLyse has been added in step 3, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless
6. Centrifuge at 12,000rpm for 5 minutes.
7. Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes.
8. Discard the flow-through in the tube. Add **750µl** of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.
9. Repeat wash procedure in step 8.
10. Discard the flow-through in the collection tube. Centrifuge at 10,000rpm for an additional minute to remove any residual Wash Solution.
11. Transfer the column to a clean 1.5ml microfuge tube. Add **50µl** of Elution Buffer into the center part of the column and incubate at room temperature for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes.
12. Store purified DNA at -20ºC.

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.

Protocol for Purification of Low Copy Plasmid DNA

EZ-10 Spin Column Plasmid DNA Minipreps Kit Components	BS4139 50 Preps	BS4149 100 Preps	BS6149 250 Preps
RNase A Solution (10mg/ml)	240µl	480µl	1.2ml
Solution I	12ml	24ml	60ml
Solution II	24ml	2X24ml	4X30ml
Solution III	2X20ml	3X25ml	6X30ml
Wash Solution	20ml	2X20ml	2X40ml
Elution Buffer	5ml	10ml	25ml
EZ-10 Column	50	100	250
Protocol	1	1	1

1. Use **5 - 10ml** overnight culture. Add overnight culture to a 1.5ml microfuge tube and centrifuge at 12,000rpm for 2 minutes. Drain the liquid completely and repeat with another portion of culture (in the same tube).
2. Add **200µl of Solution I** to the pellet, mix well and keep for 1 minute.
3. Add **400µl of Solution II** to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute. To

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 cannot remove the template and primers with chain length longer than 40-mer.

100% ethanol to 40ml Wash Solution for BS364; add 2X160ml of 96-100% ethanol to 2X40ml Wash Solution for BS664. 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).

(D) 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 5 volumes of **Buffer B3.**

Note, please ensure Isopropanol has been added to Buffer B3 prior to use.

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.

prevent contamination from genomic DNA, do not vortex.

4. Add **700 µl of Solution III**, and mix gently. Incubate at room temperature for 1minute.
5. Centrifuge at 12,000 rpm for 5 minutes.
6. Transfer half of the above supernatant (step 5) to the EZ-10 column. Let the column stand for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes. Discard the flow-through in the tube and add the second half of the supernatant, centrifuge again at 10,000 rpm for 2 minutes.
7. Discard the flow-through in the tube. Add **750µl** of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.
8. Repeat wash procedure in step 7.
9. Discard the flow-through in the collection tube. Centrifuge at 10,000 rpm for an additional minute to remove any residual Wash Solution.
10. Transfer the column to a clean 1.5ml microfuge tube. Add **50µl** of Elution Buffer into the center part of the column and incubate at room temperature for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes.
11. Store purified DNA at –20ºC.

Troubleshooting Guide: EZ-10 Spin Column Plasmid DNA Minipreps Kit

1) Low Yield
There are a number of variables that can cause low yield:

a. Each of the steps has to be strictly followed.

b. Make sure there is no precipitant in Solution I, II or III. If precipitant is present in the buffer, warm up the solution to 37°C and shake well.

c. Low culture density. Make sure that the temperature in the incubator is stable and shaking speed provides sufficient aeration of the culture.

d. Very high cell density, therefore incomplete cell lysis. Double the volume of Solution I, II and III.

2) Contamination of chromosomal DNA
Do not vortex the sample after adding solution II and III. Vigorous shaking will cause shearing of chromosomal DNA. Smaller pieces of chromosomal DNA will be captured on silica gel and carried over with purified plasmid DNA.

3) RNA contamination
RNase activity is weakened or lost. Add addition RNase A to Solution I and store at 4°C.

4) OD 260nm/280nm ratio outside 1.6-1.8 range
If the ratio of OD260nm/280nm is greater than 1.8, there may be traces of ethanol present.

If the ratio of OD260nm/280nm is smaller than 1.6, there is chance of protein and salt contamination. Make sure the sample is mixed well after Solution III is added and after spinning down. In addition, repeat one more wash step before elution step to remove extra salt completely.

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

Protocol: EZ-10 Spin Column PCR Products Purification Kit BS363, BS364, BS664

IMPROVED DESIGN FOR NANODROP USERS!

EZ-10 Spin Column PCR Purification Kit Components	BS363 50 Preps	BS364 100 Preps	BS664 250 Preps
Buffer B3[(A)(B)]	24ml	2x24ml	5x24ml
Wash Solution[(C)]	20ml	40ml	2X40ml
Elution Buffer[(D)]	5ml	10ml	25ml
EZ-10 Column	50	100	250
Protocol	1	1	1

(A) Before use, add 6ml of 96-100% of Isopropanol to 24ml Buffer B3 for BS363; add 12ml of 96-100% Isopropanol to 48ml Buffer B3 for BS364; add 30ml of 96-100% Isopropanol to 120ml Buffer B3 for BS664. For other volumes of Buffer B3, simply add enough Isopropanol to make a 1:4 ratio (volume of added Isopropanol: volume of Buffer B3 = 1:4).

(B) Dissolve the precipitate by warming the solution at 37ºC if necessary. Then cool down to room temperature before use.

(C) Before use, add 80ml of 96-100% of ethanol to 20ml Wash Solution for BS363; add 160ml of 96-