SpinColumn DNA GEL EXTRACTION MINIPREPS KIT

I. Description:

ShineGene **SpinColumn Gel Extraction Kit** offers fast DNA extraction and purification from both standard and low-melt agarose gels. Up to 20 µg of double- or single-stranded DNA can be recovered from a solubilized gel slice in just five minutes. DNA molecules of anywhere between 70 and 10,000 base pairs absorb to the Column and contaminants and inhibitors such as agarose, dyes, and salts are washed away. Eluted in a small volume of low-salt buffer, the purified DNA is immediately ready for downstream applications such as PCR, transformation, restriction enzyme digestion, cloning, sequencing, *in vitro* translation, and transfection.

II. KIT CONTENTS

Kit contains enough supplies for 50/100 minipreps.

Components	ZN00101(50Preps)(EUR50.00)	ZN00102(100Preps)(EUR80.00)
Binding Buffer II	30ml	60ml
Wash Solution (A)	12ml	24ml
Elution Buffer(B)	5ml	10ml
Column	50	100
2ml Collection Column	50	100
Protocol	1	1

(A) Before use, add 48ml of 100% of ethanol to 12ml Wash Solution for ZN00101, or add 96ml of 100% ethanol to 24ml Wash Solution for ZN00102. 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.

III. APPLICATIONS

• Recovery of DNA fragments from reaction solutions.
• Recovery of DNA fragments from agarose gels.

IV.Features:

• Quick and economical
• High yields (60-90%) of 60bp-40kb DNA fragments.
• Efficient removal of contaminants. Purified DNA can be used in any downstream application such as sequencing, labeling, restriction enzymatic digestion, ligation or transformation.
• No phenol / chloroform extraction or ethanol precipitation is required.

V.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 400ul of Binding Buffer II for each 100mg of gel weight (example – a gel slice

weighing 125mg would require 500ul of Binding Buffer II). Incubate at 50-60$_0$C for 10 minutes and shake occasionally until agarose is completely dissolved. For high concentration gels (1.5-2.0%), 700ul of Binding Buffer II per 100mg of agarose gel are added.

3. Add the above mixture to the column and let stand for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes and discard the flow-through in the tube.

4. Add 500ul of Wash Solution, and centrifuge at 10,000rpm for 2 minutes. Discard the solution in the tube.

5. Repeat step 4. Centrifuge at 10,000rpm for an additional 2 minutes to remove any residual Wash Buffer.

6. Place the column in a clean 1.5ml microfuge tube. Add 30-50ul 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 temperatures (37º to 50ºC) may slightly increase the yield. Pre-warming the Elution Buffer at 55º to 80ºC may also slightly increase elution efficiency. If a higher DNA concentration is desirable, 20ul (or less) of elution buffer can be used to elute the DNA. It is critical that the elution buffer be applied directly in the center of the column. (To recover maximum amount of DNA it is recommended to repeat the elution step.)

7. Store the purified DNA at -20ºC.

VI.Protocol for DNA purification from enzymatic reactions:

1. Transfer the volume 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 column and let stand for 2 minutes. Centrifuge at 10,000rpm for 1 minute and discard the flow-through in the tube.

3. Add 500ul 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 30-50ul 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 to the center of the column. Incubating the column with the Elution Buffer at higher temperatures may slightly increase the yield, especially of fragments larger than 10,000bp. Pre-warming the Elution Buffer at 55º to 80ºC may also slightly increase elution efficiency. If a higher DNA concentration is desirable, 20ul (or less) of elution buffer may be used. It is recommended that the elution step be repeated to recover the maximum amount of DNA.

6. Centrifuge at 10,000rpm for 1 minute to elute the DNA.

7. Store the purified DNA at -20ºC.