



PRODUCT INFORMATION

EZ-10 SPIN COLUMN DNA GEL EXTRACTION MINIPREPS KIT

Components	BS353 , 50 Preps	BS354 , 100 Preps
Binding Buffer II	30ml	2X30ml
Wash Solution	12ml	24ml
Elution Buffer	5ml	10ml
EZ-10 Column	50	100
2.0ml Collection tube	50	100

(A) Before use, add 48ml of 96-100% of ethanol to 12ml Wash Solution for BS353, or add 96ml of 96-100% ethanol to 24ml Wash Solution for BS354. 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.

Storage: The kit is stable for 12 months at room temperature. For longer storage, keep all contents of the kit cold.

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 10ug 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. DNA fragments are then eluted off the column and can be used for downstream protocols without further processing.

Application:

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

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.

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°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 EZ-10 column and let stand for 2 minutes. Centrifuge at 8,000xg for 1 minute and discard the flow-through in the tube.

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4. Add 500ul of Wash Solution, and centrifuge at 8,000xg for one minute. Discard the solution in the tube.
5. Repeat step 4. Centrifuge at 12,000xg for an additional 1-minute 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 12,000xg for 1 minute 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 (37° to 50°C) may slightly increase the yield. Pre-warming the Elution Buffer at 55° to 80°C 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 -20°C.

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 EZ-10 column and let stand for 2 minutes. Centrifuge at 5000rpm for 1 minute and discard the flow-through in the tube.
3. Add 500ul of Wash Solution, and centrifuge at 8,000rpm for 1 minute. Discard the solution in the tube.
4. Repeat step 3. Spin at 1000rpm 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 55° to 80°C 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 1000rpm for 1 minute to elute the DNA.
7. Store the purified DNA at -20°C.

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