

DNA Gel Extraction Kit

Cat. No.: A1521384 | Pack size: 50T, 100T | Storage: Room temperature

Overview

This kit is based on centrifugal spin column technology and is suitable for recovering DNA fragments from TAE or TBE agarose gels while removing impurities such as proteins, other organic compounds, inorganic salt ions, and oligonucleotide primers. Using this kit, fragments ranging from 100 bp to 15 kb can be recovered with a recovery rate of up to 80%. Each spin column can bind up to 10 µg of DNA. The purified DNA can be directly used for sequencing, ligation, restriction enzyme digestion, PCR, and transformation.

Protocol

Before use, add an appropriate amount of absolute ethanol to Buffer PW2. For 12 mL of Buffer PW2, add 48 mL of absolute ethanol; for 24 mL of Buffer PW2, add 96 mL of absolute ethanol.

1. (Optional) Column Equilibration

Place the KOS spin column into a collection tube. Add 500 µL of Buffer BL to the spin column, centrifuge at 12,000 rpm (13,680 × g) for 30 seconds. Discard the waste liquid and put the spin column back into the collection tube. (Use spin columns processed on the same day.)

Note: Treating spin columns stored at room temperature for a long time with Buffer BL can improve binding yield.

2. Cut the single target DNA band from the agarose gel (remove as much extra gel as possible) and place it into a clean centrifuge tube. Weigh the gel slice.

3. Add 3 volumes of Buffer PBD to the gel slice (if the gel weighs 0.1 g, its volume can be considered as 100 µL, then add 300 µL of Buffer PBD). Dissolve the gel at 50°C for 5–10 minutes, gently inverting the tube occasionally to ensure complete dissolution. (If the gel volume is too large, cut it into small pieces beforehand.)

Note: After the gel is completely dissolved, it is best to cool the solution to room temperature before loading onto the column, as the spin column binds DNA more efficiently at room temperature.

4. Transfer the solution from the previous step to a Spin Column KOS (place the column in a collection tube). Let stand at room temperature for 2 minutes, then centrifuge at 12,000 rpm (13,680 × g) for 30 seconds. Discard the flow-through and place the column back into the collection tube.

Note: The column has a capacity of 700 µL. If the sample volume exceeds 700 µL, load in multiple batches.

5. Add 580 µL of Buffer PW2 (ensure absolute ethanol has been added before use) to the column. Centrifuge at 12,000 rpm (13,680 × g) for 30 seconds. Discard the flow-through and place the column back into the collection tube.

Note: If the recovered DNA is intended for salt-sensitive experiments (e.g., blunt-end ligation or

direct sequencing), it is recommended to let the column stand for 2 minutes after adding PW2 before centrifugation.

6. Repeat step 5.

7. Centrifuge at 12,000 rpm (13,680 × g) for 2 minutes.

8. Place the column into a clean centrifuge tube and air-dry it with the lid open at room temperature for 2 minutes to completely evaporate any residual ethanol. Add an appropriate volume of Buffer EB to the center of the membrane (without touching it), let stand for 2 minutes at room temperature, then centrifuge at 12,000 rpm (13,680 × g) for 30 seconds to collect the DNA solution.

Note: The elution volume should not be less than 30 µL, as smaller volumes may reduce recovery efficiency. If the downstream experiment is pH-sensitive, sterile water can be used for elution. The pH of the elution buffer greatly affects elution efficiency; if using water, ensure its pH is between 7.0 and 8.5 (adjust with NaOH if necessary). A pH below 7.0 results in poor elution. To increase DNA yield, reload the eluate back onto the column and centrifuge again at 12,000 rpm (13,680 × g) for 30 seconds, collecting the DNA solution in the tube.

DNA Concentration and Purity Assessment

The purified DNA fragments can be analyzed by agarose gel electrophoresis and UV spectrophotometry to determine concentration and purity.

DNA concentration is determined by measuring the absorbance at 260 nm (A_{260}). If dilution is required, it is recommended to use Buffer EB; diluting with water may lead to inaccurate values. The accurate absorbance at 260 nm should be in the range of 0.1- 1.0. An absorbance of 1 unit at 260 nm corresponds to 50 µg/mL for double-stranded DNA and 40 µg/mL for single-stranded DNA.

The A_{260}/A_{280} ratio should be between 1.7 and 1.9. If ddH₂O is used instead of Buffer EB for elution, the ratio may be lower due to the influence of pH and ions on absorbance, but this does not indicate low purity.

Kit Contents & Storage

Product No.	Component	50T	100T	Storage
A1521384A	Buffer PBD	50 mL	100 mL	RT.
A1521384B	Buffer PW2	12 mL	24 mL	RT.
A1521384C	Buffer EB	15 mL	15 mL	RT.
A1521384D	Spin Column KOS	1 EA×50	1 EA×100	RT.
A1521384E	2 mL Collection Tube Certificates	1 EA×50	1 EA×100	RT.
A1521384F	BL buffer	30 mL	60 mL	RT.

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