AFTSpin Multifunction DNA Purification Kit

Cat. No. : RK30100

Product components

Components	Component number	Size-1 50 RXN	Size-2 200 RXN
Buffer DB	RM30102	28 mL	110 mL
Buffer W1*	RM30332	24 mL	75 mL
Elution Buffer	RM30333	15 mL	25 mL
Spin Column & Collection Tube	RM30180	50 pk	4 × 50 pk

*Note: Add 56 mL of absolute ethanol (for size 50 RXN) to Buffer W1. Add 175 mL of absolute ethanol (for size 200 RXN) to each Buffer W1 prior to initial use.

Product Description

This kit is suitable for recovering up to 10 μg DNA (80 bp~10 kb) from agarose gel or PCR products, and the recovery rate can reach 65~85%. After the agarose gel was dissolved in chaotropic salt (Buffer DB), the DNA fragments were selectively adsorbed on the silica matrix membrane in the spin column. The recovered DNA has high purity and can maintain fragment integrity and high biological activity, and can be directly used in molecular biology experiments such as sequencing, ligation, PCR amplification, and in vitro transcription.

Storage

This kit is stable when stored at room temperature.

Highlights

- Fast: the gel does not need to be weighed, and the operation is simple.
- High efficiency: high recovery efficiency and high purity of the obtained DNA.
- Wide applicability: one kit can be used for recovering DNA from agarose gel or PCR products.

Precautions

1. Prior to the initial use, add the specified amounts of absolute ethanol to the Buffer W1 and mix thoroughly. After adding, mark the reagent bottle to indicate that ethanol has been added, to avoid multiple additions!
2. Avoid volatilization, oxidation and pH changes caused by extended exposure of reagents to air. Tightly close all reagent bottles immediately after use.
3. If recovering DNA from agarose gel, a new electrophoresis buffer should be used during electrophoresis to avoid affecting the electrophoresis and recovery effect.
4. It is recommended to use high-quality agarose, so as not to affect the downstream ligation and other experiments.
5. Buffer DB contains irritating solution, wear latex gloves and glasses during operation.
6. The recovered products can be detected by agarose electrophoresis or spectrophotometer. When using a spectrophotometer, if Elution Buffer is used for elution, it is recommended to use the Elution Buffer for calibration.
7. The pH value of the eluent has a great influence on the elution efficiency. For subsequent sequencing, ddH_2O should be used for elution to ensure that the pH value is in the range of 7.0 to 8.5. If the pH value is lower than 7.0, the elution efficiency will be reduced.

Operation Description

Agarose gel recovery

1. Under a long-wave length UV lamp, slice the DNA band to be recovered from the gel with a clean blade, and remove excess gel as much as possible.

   Note: In order to avoid DNA damage caused by too long ultraviolet irradiation time, it is recommended to slice the DNA band quickly.

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2. Place the gel into a 2 mL centrifuge tube and add 500 µL Buffer DB.
3. Incubate the sample at 65°C for 4-6 minutes until the gel completely dissolves and the color becomes pale yellow. Vortex every 2-3 minutes to help accelerate dissolution.

   Note: If the gel is too large, appropriately add Buffer DB until the solution is pale yellow.
4. Add the melted gel solution to Spin Column, placed in a collection tube, and centrifuge at 12,000 rpm (~13,400 x g) for 1 minute, then discard the waste in the collection tube.
5. Add 600 µL of Buffer W1 (**check that absolute ethanol has been added**), centrifuge at 12,000 rpm (~13,400 x g) for 1 min and discard the waste.

   Note: If the recovered DNA is used for cloning experiments or direct sequencing, it is recommended to leave for 2- 5 min after adding Buffer W1 and then centrifuge.
6. Repeat Step 5 once.
7. Place the Spin Column back into the empty collection tube and centrifuge 12,000 × g for 2 min.
8. Place the Spin Column in a clean 1.5 mL centrifuge tube, open the cover and leave for 2 min at room temperature, and dry thoroughly to avoid inhibiting the downstream reaction by residual ethanol in Buffer W1.
9. Add 35-50 µL of Elution Buffer (preferably heated in a 60-65°C water bath in advance) to the center of the spin column membrane, and allow to stand at room temperature for 2 minutes. Centrifuge at 12,000 rpm (~13,400 x g) for 2 minute. To increase final sample concentration, the resulting solution can be re-added to the same Spin Column and centrifuged again for 2 minute. The resulting liquid contains highly purified DNA.

   Note: The larger the elution volume, the higher the elution efficiency. If a higher concentration of DNA is required, the elution volume can be appropriately reduced to a minimum volume of not less than 25 µL. Smaller elution volumes can reduce elution efficiency and DNA yield. DNA products should be stored at -20℃ to prevent DNA degradation.

DNA purification such as PCR products or enzyme fragments

1. Add Buffer DB (not less than 150 µL) according to the ratio of PCR product: Buffer DB= 1:3, and then mix well (for example, add 150 µL Buffer DB to 50 µL PCR product in 1.5 mL centrifuge tube, and then mix well).

   Note: If the volume of PCR product is less than 50 µL, directly add 150 µL Buffer DB.
2. Transfer the solution to the Spin Column, placed in a collection tube, and centrifuge at 12,000 rpm (~13,400 x g) for 1 minute, then discard the waste in the collection tube.
3. Add 600 µL of Buffer W1 (**check that absolute ethanol has been added**), centrifuge at 12,000 rpm (~13,400 x g) for 1 min and discard the waste.

   Note: If the recovered DNA is used for cloning experiments or direct sequencing, it is recommended to leave for 2- 5 min after adding Buffer W1 and then centrifuge.
4. Repeat Step 3 once.
5. Place the Spin Column back into the empty collection tube and centrifuge 12,000 × g for 2 min.
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