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DNA Purification Kit Instructions

Composition

DNA Purification Kit	5 Preps	50 Preps	250 Preps
Cat. No.	2101005	2101050	2101250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
Buffer P	3 ml	30 ml	75 ml×2
Buffer WB (concentrate)	1.5 ml	12 ml	60 ml
Buffer TE	0.5 ml	5 ml	25 ml
Instructions	1	1	1

Storage

1. All the reagents and components can be stored for up to 3 years at room temperature (0~30℃). For longer storage, it is recommended to keep at 2~8℃.
2. The product stored at 2~8℃ should be restored to room temperature before use.

Technical Support

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Introduction

The DNA Purification Kit is suitable for purifying up to 25 μg of high-purity DNA (100 bp-10 kb) from the reaction solution of PCR, enzymatic reaction, and sequencing reaction, with a recovery efficiency of 75%~90%, and the purified DNA does not contain primers, enzyme proteins, single nucleotides, fluorescent dyes, or radioisotope-labeled single nucleotides. Suitable for molecular biology experiments with a wide range of requirements.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 ml centrifuge tubes, pipettes, and tips.
3. Protective equipment such as disposable latex gloves and paper towels.
4. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. 3 M sodium acetate (pH 5.0) may be required.

Preparation Before Use.

1. If the centrifuge has a refrigeration function, set the temperature to 25℃.
2. Add absolute ethanol to Buffer WB according to the instructions on the label the reagent bottle and tick the box on the label to mark "Ethanol Added".

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Protocol

1. **Add 5 times the volume of Buffer P to 1 volume of the PCR product or the DNA solution that needs to be cleaned. Do not discard the tip, pipette several times to mix well, transfer the mixture to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), closed the lid.**

* For example, add 500 µl Buffer P for every 100 µl sample.

* If the PCR product contains paraffin oil, it does not need to be removed or included in the sample volume.

* DNA solutions suitable for cleaning include enzymatic reaction (e.g., digestion reactions, ligation reactions, etc.), RNase-treated DNA solutions (which can remove degraded RNA) and DNA containing impurities obtained by phenol/chloroform extraction.

* The dye added in Buffer P can indicate the pH. If the mixture turns to violet after adding Buffer P to the sample, add 10 µl 3 M sodium acetate (pH 5.0) and mix, the color of the mixture will turn yellow, otherwise the adsorption efficiency of DNA will decrease.

2. **Centrifuge at 12000 rpm for 30 sec, discard the filtrate and place the Spin Column back into the 2 ml Collection Tube.**
3. **Add 700 µl Buffer WB to the Spin Column, closed the lid, centrifuge at 12000 rpm for 30 sec. Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube.**

* Ensure that absolute ethanol has been added into Buffer WB.

4. **Centrifuge at 14000 rpm for 1 min.**

* If the centrifuge does not reach 14000 rpm, centrifuge at full speed for 2 min.

* Do not omit this step, or the residual ethanol in the eluted DNA will affects the subsequent applications.

5. **Discard the 2 ml Collection Tube, place the Spin Column into a cleaning 1.5 ml centrifuge tube, add 30~50 µl Buffer TE to the center of the column membrane, close the lid, incubate for 1 min at room temperature, and centrifuge at 12000 rpm for 30 sec.**

* The deionized water can also be used to elute DNA, but the pH should be within 7.0~8.5, otherwise it may affect the elution efficiency.

6. **Discard the Spin Column, the eluted DNA can be used for various molecular biology experiments immediately or stored at - 20℃ for later use.**

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Protocol for Proteinase K Digestion Products

1. **Add 5 times the volume of Buffer P to 1 volume of proteinase K digestion product, pipette several times to mix well, transfer the mixture to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), closed the lid.**

* For example, add 500 µl Buffer P for every 100 µl Proteinase K digestion product.
* If recovering DNA from proteinase K digestion products containing trace amounts of DNA, such as hair and blood, add an appropriate amount of carrier RNA (Simgen Cat. No. 4003101) to improve the efficiency of DNA recovery.
* If the sample is fresh and has a high DNA content, we recommend replacing the Spin Column with a genomic DNA Spin Column (Simgen Cat. No.: 7201050) to improve the efficiency of DNA recovery.

2. **Centrifuge at 12,000 rpm for 30 sec, discard the filtrate and place the Spin Column back into the 2 ml Collection Tube.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhering to the mouth of Collection Tube, you can slap the 2 ml Collection Tube upside down once on a paper towel.

3. **Add 700 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec. Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube.**

* Ensure that absolute ethanol has been added to Buffer WB.

4. **Centrifuge at 14,000 rpm for 1 min and discard 2 ml Collection Tubes.**

* If the centrifuge speed does not reach 14000 rpm, centrifuge at the full speed for 2 min.
* This step of high-speed spin dry is to remove the residual ethanol, please do not omit this step, or the residual ethanol in the eluted DNA will affects the subsequent applications.

5. **Place the Spin Column in a clean 1.5 ml centrifuge tube, add 30~50 µl Buffer TE in the Spin Column center, close the lid, incubate at room temperature for 1 min, centrifuge at 12,000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof cover, change the centrifugation conditions to 8000 rpm for 1 min to avoid the tube lid falling off and damaging the centrifuge.
* If DNA is purified with genomic DNA Spin Columns, add 100-200 µl Buffer TE to elute DNA.
* DNA can also be eluted with deionized water, but make sure that the pH of the deionized water used is within 7.0~8.5, otherwise it will cause the low DNA elution efficiency.

6. **Discard the Spin Column, the eluted DNA can be used immediately for various molecular biology experiments or stored at -20℃ for later use.**