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Long Fragment DNA Selection Kit Instructions

Composition

Long Fragment DNA Selection Kits	50 Preps	250 Preps
Cat. No.	2105050	2105250
Spin Columns	50	250
2 ml Collection Tubes	50	250
Buffer P5	30 ml	75 ml×2
Buffer WB (concentrate)	12 ml	60 ml
Buffer TE	5 ml	25 ml
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Storage

1. All the reagents and components can be stored for up to 2 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 kit is suitable for recovery of up to 20 μg of high-purity DNA (200 bp-10 kb) from PCR-amplified reactions, with recovery efficiencies between 70-80%, and no primers, enzyme proteins, single nucleotides, fluorescent dyes, or radioisotope-labeled single nucleotides. It is 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 tubes, pipettes, and tips.
3. Disposable gloves, tissues and protective equipment.
4. Microcentrifuge (s)(with rotor for 1.5 ml and 2 ml centrifuge tubes).
5. 3M sodium hydroxide may be required.

Preparation Before Use

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

Protocol

1. **Add 5 volumes of Buffer P5 to the PCR product or DNA solution to be cleaned, do not discard the tip, pipette a few times to mix well, and transfer the mixture to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube) and close the cap.**

* For example, add 500 µl Buffer P5 for every 100 µl sample.
* If you want to recover DNA from PCR products larger than 120 µl, transfer the mixture to a Spin Column in several times (the maximum volume of the Spin Column is 750 µl) and follow step 2 until the mixture has fully filtered through the Spin Column.
* The dye added to Buffer P5 indicates the pH value, and the mixed solution should be orange-red when Buffer P5 is added. If the mixed solution turns yellow after adding Buffer P5, the DNA solution that needs to be cleaned is too acidic, and 1-5 µl of 3 M NaOH should be added to restore the original orange-red color of the mixed solution, otherwise the removal of DNA fragments below 200 bp will be affected.

2. **Centrifuge at 12000 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 adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

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 Spin Column center, close the lid, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof cover, it is recommended to change the conditions to centrifuge at 8000 rpm for 1 min to prevent the 1.5 ml centrifuge tube lid from falling off and damaging the centrifuge.
* Do not elute DNA with less than 30 µl of Buffer TE, as this will not help increase the concentration of eluted DNA because the membrane of the Spin Column is not sufficiently wetted but will reduce the efficiency of DNA recovery.
* 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°C for later use.**