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MinElute DNA Gel Extraction Kit Instructions

Composition

MinElute DNA Gel Extraction Kit Cat. No.	5 preps 2002005	50 preps 2002050	250 preps 2002250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
1.5 ml Collection Tubes	5	50	250
Buffer G	3 ml	30 ml	150 ml
Buffer WS	3 ml	30 ml	150 ml
Buffer WG (concentrate)	2 ml	17 ml	40 ml×2
Buffer TE	0.5 ml	5 ml	25 ml
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Storage

1. All the reagents can be stored at room temperature for up to 3 years without showing any reduction in performance and if stored at 2~8℃, it would be stable for more than 3 years.
2. Products stored at 2~8 °C should be restored to room temperature before use.

Technical Support

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Introduction

This kit uses the column purification method, and the specially designed Spin Column ensures that concentrated DNA can be efficiently eluted with micro volumes as low as 10 µl, which is suitable for recovery of up to 10 µg of DNA (70 bp-10 kb) from up to 400 mg agarose gels, with a recovery rate of up to 80~90%. The mild dissolved buffer without sodium iodide to ensure that the recovered DNA maintains fragment integrity and high biological activity, and the recovered DNA can be directly used for molecular biology experiments such as ligation, in vitro transcription, PCR amplification, sequencing, and microinjection.

Equipment And Reagents to Be Supplied by Users

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 cntrifuge tubes).
5. 3 M sodium acetate (pH 5.0), isopropanol may be required.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Add absolute ethanol to Buffer WG according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol Added".
3. Set the water bath temperature to 50℃.

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Protocol

1. **Cut the agarose gel containing the DNA fragments under UV light and transfer to a 1.5 ml centrifuge tube (not provided).**

* Minimize the size of the gel slice by removing extra agarose to reduce dissolve time.

2. **Weigh gel and add 3×volumes of Buffer G (1 µl gel volume per 1 mg gel).**

* e.g., Add 300µl Buffer G to 100 mg agarose gel.
* For >2% agarose gels, 6×volumes of Buffer G should be added.

3. **Incubate the tube at 50°C until the gel is completely dissolved (approximately 5-10 min).**

* Flip the tube every 2-3 min during the incubation to help the gel dissolve and observe whether the gel dissolves completely.
* The dye added to Buffer G can help to see if the gel is completely dissolved and can also indicate the pH value, if the solution is turns to violet, add 10 µl 3 M sodium acetate (pH 5.0) to restore the solution to its original color, otherwise it will affect the binding of DNA to the cartridge.

4. **Add 1×the gel volume of isopropanol and mix well.**

* If the recovered DNA fragment is between 500 bp~4 kb, this step can be omitted.
* For example, to recover DNA from a 100 mg gel, 100 µl isopropanol should be added.
* If DNA is recovered from a gel greater than 2%, 2 times the gel volumes of isopropanol should be added.

5. **Transfer the mixture to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube) and centrifuge at 12,000 rpm for 30 sec.**

* If the volume of mixture is greater than 800 µl, divide the mixture in two separate centrifuge steps.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WS, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* 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.

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

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

8. **Repeat step 7 once.**

* Two Buffer WG washes are more effective at reducing salt carryover on the cartridge and should not be omitted.

9. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at its full speed for 2 min.
* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol and the subsequent experimental results may be affected.

10. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml collection tube, add 10-20 µl Buffer TE to the Spin Column center, close the lid, incubate at room temperature for 1 min, and centrifuge at 12,000 rpm for 30 sec to elute the DNA.**

* DNA can also be eluted with deionized water but ensure that the pH of the deionized water used is 7.0-8.5, otherwise the elution efficiency of the DNA will be affected.
* Do not elute DNA with less than 10 µl Buffer TE, as this will not help increase the concentration of the eluted DNA because the membrane of the cartridge is not sufficiently wetted and will reduce the efficiency of DNA recovery.

11. **Discard the Spin Column, and the eluted DNA can be immediately used in various molecular biology experiments or stored at -20°C for later use.**