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DNA Gel Extraction Kit (10-50 kb) Instructions

Composition

DNA Gel Extraction Kit (10-50 kb) Cat. No.	5 Preps 2003005	50 Preps 2003050	250 Preps 2003250
Buffer SI	180 µl	1.8 ml	9 ml
Buffer G	3 ml	30 ml	150 ml
Buffer WS	3 ml	30 ml	150 ml
Buffer WG (concentrate)	2.2 ml	25 ml	40 ml×3
Buffer TE	0.5 ml	5 ml	25 ml
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Storage

1. All the reagents can be stored at room temperature (0~30℃) for up to 3 years without showing any reduction in performance and if stored at, 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 is suitable for DNA recovery (10-50 kb) from up to 400 mg agarose gels with a recovery rate of 50~75%. The mild dissolved buffer without sodium iodide to ensure that the recovered DNA maintains fragment integrity and high biological activity and can be directly used in molecular biology experiments such as ligation, PCR amplification, and sequencing.

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 centrifuge tubes).
5. Vortexer and water bath.
6. 3 M sodium acetate (pH 5.0) may be required.

Preparation before use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Set the water bath temperature to 50℃.
3. 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".

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Protocol

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

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

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

* e.g., Add 200µl Buffer G to 200 mg agarose gel.

3. **Vortex and shake Buffer SI to suspend all the media in it and transfer 30 µl Buffer SI into the 1.5 ml centrifuge tube containing the gel.**
4. **Incubate the tube at 50°C until the gel is completely dissolved (about 5~10 min).**

* Invert the tube every 2-3 min during the incubation to help the gel dissolve and observe whether the gel dissolves completely.
* After observing the dissolution, flick the tube wall to suspend the white medium to mix well.
* The dye added to Buffer G can help to observe if the gel is completely dissolved and can indicate the pH, if the solution turns violet, 10 µl 3 M sodium acetate (pH 5.0) should be added to restore the solution to its original color, otherwise it will affect the recovery efficiency of DNA.

5. **Centrifuge at 8,000 rpm for 30 sec and carefully discard the supernatant with a tip.**

* Be careful not to discard the pellet from the bottom of the tube, which contains DNA.

6. **Add 500 µl Buffer WS, close the lid, gently invert the tube until the pellet is completely dispersed.**

* This step is to remove residual trace amounts of agarose molecules.
* This step should not be shaken by vortexing, otherwise it may lead to the fragmentation of large DNA fragments; If the pellet is not easily dispersed, you can flick the tube wall to disperse it.

7. **Centrifuge at 8,000 rpm for 30 sec and carefully discard the supernatant with a tip.**
8. **Add 1 ml Buffer WG, close the lid, gently invert the tube to disperse the pellet. Centrifuge at 8,000 rpm for 30 sec and carefully discard the supernatant with a tip.**

* Ensure that absolute ethanol has been added to Buffer WG.
* This step should not be shaken by vortexing, otherwise it may lead to the fragmentation of large DNA; If the pellet is not easily dispersed, you can flick the tube wall to disperse it.
* Step can be omitted: Repeat step 8 once. Repeat the wash once ensures that the DNA with lower salt residue is obtained.

9. **Close the lid and spin down the solution to the bottom of the tube. Carefully discard the supernatant as much as possible with a 200 µl tip. Allow to dry the pellet at room temperature.**

* The pellet should be in the form of a white powder when drying.
* Centrifuge tubes can be placed in a 37°C incubator to speed up drying, but negative pressure drying should not be used, as negative pressure drying can lead to over-drying and affect the elution efficiency of DNA.

10. **Add 20~30 µl Buffer TE, flick the centrifuge tube to disperse the pellet, and incubate at 50°C for 10 min.**

* 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.

11. **Centrifuge at 12,000 rpm for 30 sec and carefully transfer the supernatant containing DNA into another clean centrifuge tube. The obtained DNA can be immediately used in various molecular biology experiments or stored at -20°C for later use.**