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Poly-Gel DNA Extraction Kit Instructions

Composition

Poly-Gel DNA Extraction Kit Cat. No.	5 Preps 2005005	50 Preps 2005050	250 Preps 2005250
Grinding Rods	5	50	250
Filter Columns	5	50	250
2 ml collection tubes	5	50	250
Buffer PIS	1.8 ml	18 ml	90 ml
Buffer AE	3.6 ml	36 ml	180 ml
Buffer PW	6 ml	60 ml	150 ml×2
Buffer TE	0.5 ml	5 ml	25 ml
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Storage

If the kit is stored at room temperature (0~30℃), it can keep the performance unchanged for 3 years, and if stored at 2~8℃, the validity period can be extended to more than 3 years (the kit stored at 2~8℃ should be restored to room temperature before use).

Technical Support

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Introduction

The kit uses the principle of precipitation to recover single- and double-stranded DNA of various lengths from polyacrylamide gels up to 300 mg, and the recovered DNA can be directly used in molecular biology experiments such as ligation reactions, PCR, Southern hybridization, etc.

Equipment And Reagents to Be Supplied by Users

1. Pipettes and tips.
2. Disposable gloves, tissues and protective equipment.
3. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml tubes).
4. Water bath

Preparation before use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Set the temperature of the water bath to 65℃.

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Protocol

1. **Cut the polyacrylamide gel containing the DNA fragment of interest, transfer it to a 1.5 ml centrifuge tube (not provided), and grind the gel well with a grinding rod.**

* Minimize excess gel volume and keep gel weight below 0.3 g.

2. **Add 300 µl Buffer PIS, incubate at 65℃ for 1-4 h.**

* The incubation time depends on the size of the gel block and the size of the DNA fragment. Elution efficiencies can reach up to around 70% after 4 h incubation at 65℃ for DNA fragments of 100 bp size, and longer incubation times for larger gel blocks and longer DNA fragments.
* If the gel is larger and weighs more than 0.3 g, the amount of Buffer PIS can be increased until the gel fragments are completely submerged.

3. **Transfer the broken gel and DNA elution to a Filter Column and centrifuge at 12,000 rpm for 5 min.**

* Gel fragments can be transferred into the Filter Column with tips.
* If the gel weight exceeds 0.3 g, the Filter Column should be filtered in fractions, and the filtrate should be transferred to a centrifuge tube (not provided) after each filtration.

4. **Transfer the filtrate to the 1.5 ml centrifuge tube (not provided), add 600 µl (2 ×volume) of Buffer AE, close the lid, and invert to mix well.**

* Incubate at -20°C for 30 min to help DNA precipitation.

5. **Centrifuge at 12,000 rpm for 15 min and carefully discard the supernatant with tips.**

* When placing the tube in the centrifuge, it is best to place the plastic stem of the tube facing outward, so that the sediment always collects on the inner wall of the tube farthest from the center of the rotor, which is the side of the stem. Knowing where the DNA precipitate is, it is easier to find the visible precipitate, even if the precipitate is not visible due to the small amount of DNA, you can know where it is, so that you can stick the tip to the other side to operate and avoid taking the precipitate away when aspirating and discarding the supernatant. Subsequent aspiration and discarding of the supernatant step are the same as above, and careful operation is carried out to preserve the DNA pellet from the bottom and wall of the tube as much as possible.

6. **Add 1 ml Buffer PW, close the lid, and resuspend the pellet. Centrifuge at 12,000 rpm for 5 min and carefully discard the supernatant with tips.**
7. **Spin down the solution to the bottom of the tube, and carefully discard the residual liquid with a 200 µl tip. Open the lid and incubate at room temperature for 5-10 min to dry.**
8. **Add 15~30 µl Buffer TE to dissolve the DNA pellet.**
9. **The dissolved DNA can be immediately used in various molecular biology experiments or stored at -20℃ for later use.**