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DNA Gel Extraction Kit Instructions

Composition

DNA Gel Extraction Kit Cat. No.	5 Preps 2001005	50 Preps 2001050	250 Preps 2001250
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
Instructions	1	1	1

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 would be stable more than 3 years if stored at 2~8℃.
2. Reagents stored at 2~8℃ should be restored to room temperature before use.

Technical Support

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Introduction

The DNA Gel Extraction Kit is suitable for extract up to 15 μg of DNA (70 bp-10 kb) from ordinary/low melting agarose gels up to 500 mg prepared with TAE or TBE buffer with a recovery rate of 70%~85%. The mild dissolved buffer without sodium iodide to ensure that the recovered DNA maintains fragment integrity and high biological activity. Buffer WS and Buffer WG washes minimizes salt residues in the recovered DNA and ensures that it can be directly used in molecular biology experiments such as ligation, in vitro transcription, PCR, sequencing, and microinjection.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 ml centrifuge tube, pipette, 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. Water bath.
6. Isopropanol, 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 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 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.
* The gel weight should not exceed 500 mg, otherwise it will block the Spin Column or affect the DNA recovery efficiency.

2. **Add 500 µl Buffer G, incubate at 50℃ until gel is completely dissolved (about 10 min).**

* The dissolution time will increase as the volume of the gel increases. For >2% agarose gels, more dissolution time is required.
* Invert the tube several times every 2-3 min to help dissolve gel.
* Check that the color of the mixture is yellow (like Buffer G without dissolved agarose). If the color of the mixture is violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.
* If more than 300 mg was used, it will be difficult to judge whether the gel is completely dissolved. Use a pipette to aspirate the liquid carefully and observe if there is any residual gel.

3. **Add 200 µl isopropanol and mix thoroughly.**

* If the DNA fragment is between 500 bp ~ 4 kb, this step can be passed over.

4. **Transfer the mixture into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), Centrifuge at 12000 rpm for 30 sec. Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube.**

* For the mixture volumes of more than 800 µl, it should be centrifuged through the Spin Column twice.
* If the gel weight exceeds 300 mg or the concentration is ≥2%, maybe it needs to be centrifuged at full speed for 1 min again.

5. **Add 500 µl Buffer WS to the Spin Column, closed the lid and centrifuge at 12000 rpm for 30 sec. Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube.**

* This step is to remove the residual trace agarose molecules. If the recovered DNA is not for sequencing, in vitro transcription or microinjection experiments, this step can be passed over.

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

* If the recovered DNA is used for salt sensitive experiments, such as blunt-end ligation experiment or sequencing, it is recommended to add Buffer WG and incubate at room temperature for 2-5 min before centrifugation.
* Ensure ethanol has been added into Buffer WG.

7. **Repeat step 6 once.**
8. **Centrifuge at 14000 rpm for 1 min.**

* If the maximum speed of the centrifuge could not up to 14000 rpm, centrifuge at full speed for 2 min.
* Do not pass over this step, or the residual ethanol in the eluted DNA will affects the final applications.

9. **Discard the 2 ml Collection Tube, place the Spin Column into a cleaning 1.5 ml Collection Tube, add 25-30 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.**

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

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

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Protocol for Larger Than 500 mg Agarose Gel Sample

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).**

* If the gel block is large, the gel block can be minced to speed up subsequent gel dissolution.

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

* For example, 600 mg gel should be added with 1.8 ml Buffer G.
* If the gel concentration is greater than 2%, 6×volume of Buffer G should be added.
* Buffer G（Cat. No.: B107030) can be ordered separately.

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

* Invert the tube several times every 2-3 min during the incubation to help the gel dissolve and observe if the gel is completely dissolved.
* The dye added in Buffer G can help to observe whether the gel is completely dissolved and at the same time indicates the pH value, if the solution turns violet, 10 µl of 3 M sodium acetate (pH 5.0) should be added to restore the solution to yellow, otherwise it will affect the binding of DNA to the Spin Column.

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

* If the recovered DNA fragment is between 500 bp~4 kb, this step can be omitted.
* For example, to recover DNA from a 600 mg gel, 600 µl of isopropanol should be added.
* If DNA is recovered from a gel greater than 2%, 2 times the gel volume of isopropyl alcohol should be added.

5. **Transfer 800 µl mixture into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube). Centrifuge at 12,000 rpm for 30 sec, Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube.**
6. **Repeat step 5 several times until all the remaining mixture filtered through the Spin Column.**

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

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

* This step can be omitted if the recovered DNA is not used for sequencing, in vitro transcription, or microinjection experiments to remove residual trace agarose molecules.

8. **Add 700 µl Buffer WG, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* If the recovered DNA is for salt-sensitive experiments, such as flat-end ligation experiments or direct sequencing, it is recommended to incubate at room temperature for 2-5 min after adding Buffer WG before centrifugation.
* Ensure ethanol has been added into Buffer WG.

9. **Repeat step 8 once.**
10. **Discard the filtrate and place the Spin Column back in the 2 ml Collection Tube. Centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge speed does not reach 14000 rpm, centrifuge at the full speed for 2 min.
* Do not pass over this step, otherwise it may affect the subsequent experimental effect due to the ethanol mixed with the purified nucleic acid.

11. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml Collection Tube, add 25-30 µl of 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 DNA is eluted with deionized water, ensure that the pH of the deionized water used is within 7.0-8.5, otherwise the elution efficiency of the DNA will be affected.

12. **Discard the Spin Column, the eluted DNA can be used for molecular biology experiment immediately or stored at -20°C for later use.**

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Protocol for Clean Recovered DNA

1. **Add 3× volume of Buffer G to the PCR product or DNA solution that needs to be cleaned.**

* For example, if 100 µl PCR product needs to be cleaned, 300 µl of Buffer G should be added.
* If the PCR product contains paraffin oil, it does not need to be removed and does not need to be included in the volume of the sample.
* DNA solutions suitable for cleaning include enzymatic reaction solutions (e.g., digestion reactions, ligation reactions, etc.), RNase-treated DNA solutions (to remove degraded RNA), and DNA obtained after phenol/chloroform extraction.
* The dye added in Buffer G indicates pH, if the solution is turns to violet after adding Buffer G to the sample, 10 µl 3M sodium acetate (pH 5.0) should be added to restore the solution to its original color, otherwise it will affect DNA binding to the Spin Column.

2. **Add 1× the DNA solution volume of isopropanol and mix well.**

* If the cleaned DNA fragment is between 500 bp~4 kb, this step can be passed over.
* For example, if 100 µl PCR product needs to be cleaned, 100 µl isopropanol should be added.

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

* If the mixture volume is greater than 800 µl, centrifuge the column in two parts.

4. **Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WS, close the lid. 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 adhering to the nozzle of the centrifuge tube, you can slap the 2 ml Collection Tube upside down once on a paper towel.

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

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

6. **Repeat step 5 once.**

* Two Buffer WG washes can more effectively reduce salt residue on the Spin Column, do not passed over.

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

* If the centrifuge speed does not reach 14000 rpm, centrifuge at the full speed for 2 min.
* Do not pass over this step, otherwise it may affect the subsequent experimental effect due to the ethanol mixed with the purified nucleic acid.

8. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml Collection Tube, add 25-30 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.**

* 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 the elution efficiency of the DNA will be affected.

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