Molecular cloning and genetic engineering – DNA Gel Recovery Kit

Aim

The kit is suitable for recovering up to 8 μg DNA (70bp-10Kb) from all kinds of agarose gels, with a recovery rate of 60-85%. Agarose gel is melted in mild buffer (DE-A solution), where the protectant inside could prevent the degradation of linear DNA at high temperature, and then DNA is selectively bound to the membrane with the presence of DEB solution. Purified DNA has high purity, remain fragment integrity and high bioactivity. It can be directly used in molecular biological experiments such as ligation, transcription in vitro, PCR amplification, sequencing and microinjection.

Materials

Cat. No.	AP-GX-4	AP-GX-50	AP-GX-250
Preparation times	4 Preps	50 Preps	250 Preps
Preparation tube	4	50	250
2 ml centrifuge tube	4	50	250
1.5 ml centrifuge tube	4	50	250
Buffer DE-A	6 ml	2* 33 ml	2* 165 ml
Buffer DE-B	3 ml	33 ml	165 ml
Buffer W1	2.8 ml	28 ml	135 ml
Buffer W2 concentrate	2.4 ml	24 ml	2* 72 ml
Eluent	1 ml	5 ml	25 ml
Instruction	1	1	1

Buffer DE-A: Gel melting agent, containing DNA protectant to prevent DNA

degradation at high temperature. Stored in airtight condition at room temperature. Buffer DE-B: Binding solution (promotes DNA fragments greater than 70bp to bind to DNA preparation membranes selectively). Stored in airtight condition at room temperature. Buffer W1: Washing solution, stored in airtight condition at room temperature. Buffer W2 concentrate: desalting solution, add absolute ethanol (100% ethanol or 95% absolute ethanol) according to the volume specified on the reagent bottle before use, mix well and store in airtight condition at room temperature. Eluent: Eluent, stored in airtight condition at room temperature.

Procedure

1. Preparation for Experiments

(1) Before the first use, absolute ethanol of specified volume should be added to Buffer W2 concentrate.

(2) Prepare Tip head and centrifuge tubes which are nucleic acid and nuclease free.

(3) Prepare a 75℃ water-bath.

(4) Before use, check whether there is precipitation in Buffer DE-B. If there is precipitation, it should be heated in 70℃ warm water-bath until the precipitation is completely dissolved and cooled down to room temperature before use.

2. Operation Steps

(1) Cut off the agarose gel containing the target DNA under ultraviolet lamp. Use tissue to drain the gel surface and chop it up. Weigh the gel, (the weight of a 1.5 ml centrifuge tube should be recorded in advance) and the weight measured serve as a gel volume (e.g. 100 mg = 100 μl volume).

(2) Add Buffer DE-A of 3 gel volume. Heat the mixture at 75℃ (low melting-point agarose gel be heated at 40℃), intermittently mix (2-3 min) until the gel tile is completely melted (about 6-8 min).

(3) Add Buffer DE-B of 0.5 Buffer DE-A volume, mix the solution. When the DNA fragment to be separated is less than 400bp, isopropanol of 1 gel volume need to be added.

* After adding Buffer DE-B, the color of the mixture turns yellow, and the mixture should be fully mixed to ensure a well-distributed yellow solution.

Step 4-6 can choose negative pressure method or centrifugal method.

A. Negative pressure method

4A. Connect the negative pressure device correctly and insert the DNA preparation tube into the outlet of the negative pressure device. Transfer the mixture from step 3 to the preparation tube, open and adjust the negative pressure to -25-30 inch/Hg, and slowly remove the solution from the tube. 5A.

Add 500 ml Buffer W1 and remove the solution in the tube.

6A. Add 700 μL Buffer W2 and remove the solution in the tube. Wash again with 700 μL Buffer W2 in the same way.

* Make sure that absolute ethanol has been added to the Buffer W2 concentrate according to the specified volume of the reagent bottle.

* Washing with Buffer W2 two times ensures that the salt is completely removed, eliminating the impact on subsequent experiments.

7A. The preparation tube was placed in a 2 ml centrifuge tube (provided by the kit) and centrifuged for 1 minute at 12,000 x g.

B. Centrifugal method

4B. Transfer the mixture in step 3 to the DNA preparation tube (placed in a 2 ml centrifugal tube provided in the kit) for centrifugation for 1 min at 12,000 x g. Discard the filtrate.

5B. Put the preparation tube back into a 2 ml centrifuge tube, add 500 ml Buffer W1, centrifuge for 30 s at 12,000 x g. Discard the filtrate.

6B. Put the preparation tube back into 2 ml centrifugal tube, add 700 ml Buffer W2, then centrifuge for 30 seconds at 12,000 x g, discard the filtrate. Wash again with 700 ml Buffer W2 in the same way, then centrifuge for 1 min at 12,000 x g.

* Make sure that absolute ethanol has been added to the Buffer W2 concentrate according to the specified volume of the reagent bottle.

* Washing with Buffer W2 two times ensures that the salt is completely removed,, eliminating the impact on subsequent experiments.

7B. Put the tube back in a 2 ml centrifugal tube and centrifuge for 1 min at 12,000 x g.

8. Place the preparation tube in a clean 1.5 ml centrifugal tube (provided in the kit). Add 25-30 ml Eluent or deionized water in the center of the preparation tube and place it at room temperature for 1 minute. DNA was eluted by centrifugation for 1 minute at 12,000 x g.

* Heating Eluent or deionized water to 65℃ will improve the elution efficiency.

Note

1. Buffer DE-A (containing β-mercaptoethanol), Buffer DE-B and Buffer W1 contain irritant compounds. Wear latex gloves and glasses when operating. Avoid contact with skin, eyes and clothes. Beware of inhalation of mouth and nose. If skin or eyes are contaminated, rinse them immediately with plenty of water or saline, and seek medical advice if necessary.

2. In step 1, the gelatin is suggested to cut into small fragments, which will

greatly reduce the gel melting time (linear DNA is easy to hydrolyze for a long time exposure to high temperature conditions), therefore improving the recovery rate. Do not expose the gel containing DNA to ultraviolet lamp for a long time in order to reduce the damage caused by ultraviolet radiation to DNA.

3. In step 2, the gel must be completely melted, otherwise it will seriously affect the recovery rate of DNA.

4. Heating Eluent or deionized water to 65 ℃ is beneficial to improve the elution efficiency.

5. The DNA molecule is acidic and is recommended to be stored in 2.5 mM Tris-HCl and pH 7.0-8.5 eluent.