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LiPure™ Gel Extraction Kit

Cat. #: M0035 Size: 200 rxn

Introduction

LiPure™ Gel Extraction Kit utilizes a new silica membrane technology and reagent formulation to recover 100 bp to 10 kb of DNA from agarose gels. This kit uses a unique spin column to bind DNA, followed by washing and eluting steps. The rate of gel dissolving is fast, and the recovery efficiency is high.

The solution contains pH indicator, which can be used to determine if the gel recovery is optimal. Each column can adsorb up to 10 μg of DNA, while effectively removing impurities such as primers, enzymes, mineral oil, and agarose. The recovered DNA is high in purity and concentration and has good integrity. The DNA can be directly used for molecular biology experiments such as sequencing, ligation, transformation, labeling, and in vitro transcription.

Package Information

Components	M0035
Buffer PG	100 ml
Buffer PS	60 ml
Buffer PW (concentrated)	50 ml
Buffer EB	30 ml
Spin Columns DM with Collection Tubes	200

Not included in the kit: 100% ethanol; Isopropanol.

Storage

Store at Room temperature (15-30°C)

Preparation before the experiment and precautions

1. 100% ethanol should be added to the Buffer PW before the first use according to the instructions on the reagent bottle label.

2. Please check the Buffer PG before use. If crystallization or precipitation occurs, it can be placed in a 37°C water bath for 3-5 minutes to become clear.

3. It is better to use new running buffer during electrophoresis. If the downstream experiment requires a higher level, please try to use TAE buffer.

4. When cutting the gel, the exposure time to UV should be as short as possible to avoid DNA damage.

5. The recovery efficiency is related to the initial amount of DNA and the elution volume. The smaller the initial amount, the smaller the elution volume and the lower the recovery efficiency.

6. Preheat the water bath to 50°C.

7. Buffer PG contains pH indicator. When the pH is ≤ 7.5, the color of the solution is yellow. At this time, the DNA can effectively bind with the membrane. When the pH is too high, the color of the solution turns orange and purple and needs to be adjusted.

8. All centrifuge steps can be done at room temperature.

Protocol

1. Cut the single DNA band from the agarose gel; Place it in a clean centrifuge tube (self-prepared), and weight the gel (weight the centrifuge tube first)

Note: If the size of the gel is too big, cut it into pieces.

2. Add 1 volume of Buffer PG to the tube (eg, for a 100 mg gel, add 100 μl Buffer PG).

3. Incubate the tube in a 50°C water bath and gently invert the tube every 2-3 minutes until the solution is yellow to ensure that the gel is fully dissolved. If there is undissolved gel, add more Buffer PG and wait for a few more minutes until the gel is completely dissolved.

Note: 1) After the gel is completely dissolved, the solution should be yellow and proceed the experiment; if the solution is orange or purple, add 10-30 μl of 3 M sodium acetate (pH 5.0) to adjust the color to yellow before proceeding.

2) After the gel is completely dissolved, it is better to wait the temperature of the solution back to room temperature and then load it to the column. The capacity of the column to bind DNA at a relatively high temperature is weak.

4. (Optional) If the DNA fragment is < 300 bp, add 1⁄2 volume of isopropanol and mix by upside down (if the gel is 100 mg, add 50 μl of isopropanol).

5. Column equilibration: Add 200 μl Buffer PS to the column (Spin Columns DM) with the collection tube; centrifuge at 13,000 rpm (~16,200× g) for 1 minute; discard the waste from the collection tube and put the column back to the collection tube.

6. Transfer the solution obtained in step 3 or 4 to a column with collection tube; leave it at room temperature for 2 minutes, and centrifuge at 13,000 rpm for 1 minute; Discard the waste in the collection tube and put the column back to the collection tube.

Note: The maximum volume of the column is 750 μl. If the sample volume is greater than 750 μl, it can be added by multiple times.

7. Add 450 μl Buffer PW to the column (check if 100% ethanol has been added before use). Centrifuge for 1 minute at 13,000 rpm. Discard the waste from the collection tube and return the column to the collection tube.

Note: If the recovered DNA is used for salt-sensitive experiments (such as blunt-end ligation or direct sequencing), it is recommended to add Buffer PW and let it stand for 2-5 minutes before centrifugation.

8. Repeat step 7.

9. Centrifuge at 13,000 rpm for 1 minute and discard the waste from the collection tube.

Note: The purpose of this step is to remove the residual ethanol in the column, and the residual of ethanol will affect the downstream enzymatic reactions (enzyme digestion, PCR, etc.).

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10. Place the column in a new 1.5 ml centrifuge tube (selfprepared) and add 50 µl of Buffer EB in the middle of the membrane and allow it to stand at room temperature for 2 minutes. Centrifuge at 13,000 rpm for 1 minute and collect the DNA solution. Store the DNA at -20°C.

Note: 1) In order to increase the amount of DNA recovered, the solution obtained can be added back to column, and leave it at room temperature for 2 minutes, and centrifuge at 13,000 rpm for 1 minute.

2) The elution volume should not be less than 30 µl. Too little volume will affect recovery efficiency.

3) When DNA fragments are larger than 10 kb, Buffer EB should be preheated in a 50°C water bath to increase recovery efficiency.

Note: This kit is also suitable for the purification and recovery of PCR products. Add an equal volume of Buffer PG to the PCR product and mix thoroughly (for fragments smaller than 150 bp, increase to 3 times of the volume to increase the recovery rate). Follow the step 5 above for subsequent operations.