Gel DNA Purification Kit (Catalog: TBS6033-200)

Tribioscience's Gel DNA Product Purification Kit provides a fast and easy method for the reliable purification of DNA from standard or low-melting agarose gel in a TAE or TBE buffer system. In this kit, a glass fiber membrane is used to recover DNA from 80 bp to 100 kb. The extracted DNA can reach 85% of the yield rate. No organic extraction and alcohol precipitation is required, and multiple samples can be easily processed simultaneously.

Kit Components

Kit components	TBS6033-200
Number of Preparation	200
Binding Buffer	200 mL
Concentrated Wash Buffer*	50 mL
Elution Buffer	30 mL
MiniSpin Columns with Collection Tubes	200
Collection Tubes （1.5 mL）	200

Note: *Required to add indicated absolute ethanol before the first-time use as below.

Preparation

1. All centrifugations should be carried out at 10,000 x above (>12,000 rpm) at room temperature in a microcentrifuge.
2. All solutions should be equilibrated at room temperature before use.
3. Prepare water bath or heating block to 50˚C.
4. For large fragments (>5 kb), pre-warm Elution Buffer to 70˚C.
5. Add 200 mL of absolute ethanol into **50 mL concentrated Wash Buffer** to make 250 mL working solution Before the first-time use.

Protocols

1. Excise the DNA band of interest using an ethanol-cleaned razor blade or scalpel on a transilluminator.
   Notes: Minimize gel volume by cutting the gel slice as small as possible.
2. Weigh the gel slice in a microcentrifuge tube. Add 3 volumes (µL) of **Binding Buffer** to 1 volume (mg) of gel.
   Notes: For 100 mg agarose gel slice, add 300 µL *of binding buffer. For >1.5 % agarose gel, add 5 volumes of Binding buffer.*
3. Incubate at 50°C until the agarose gel is completely melted (5 ~ 10 min).
4. After the slice has dissolved completely, check that the color of the mixture is yellow (similar to Binding Buffer).
   Notes: If the color of the mixture becomes brown or purple, add 10 µL of 3M sodium acetate, pH 5.0, and mix.
5. (**Optional :**) Add 1 gel volume of isopropanol to the sample and vortex to mix. For 100 mg of gel volume, add 100 µl of isopropanol. (*Note: Do NOT centrifuge at this step. This step is required to increase the recover yields of DNA fragments 5 kb. For the DNA fragments between 200 bp and 5 kb, it has little effect on the recovery.*)
6. Transfer the mixture to a MiniSpin Column with collection tube. Centrifuge for 1 min. Discard the pass-through and re-insert the column into the Collection Tube.

7. (**Optional :**) Apply 500 ul of Binding Buffer to the column. Centrifuge for 30 sec. Discard the pass-through and reinsert the column into the Collection Tube. This step is for further complete removal of any traces of agarose and required only for direct use of purified DNA for very sensitive applications, such as in vitro transcription. Usually, this step can be skipped for automatic sequencing or ligation
8. Apply 700 µL of **Washing Buffer**. Centrifuge for 30 sec. Discard the passthrough and reinsert the column back into the collection tube.
9. Centrifuge for an additional 1 min to remove residual wash buffer. Transfer the column to a new 1.5 ml tube.
10. Apply 50 µL of **Elution Buffer** or ddH2O to the center of the membrane in the column, let stand for 1 min, and centrifuge for 1 min.
    Notes: To obtain more concentrated DNA solution, apply 30 µL *of elution buffer, but the volume lower than 30* µL *will decrease the yield significantly. Up to 200* µL *of elution buffer can be applied to MiniSpin column, and it will reduce the concentration of DNA.*