DONGSHENG BIOTECH

Item No: **N1081** **100 preps**
N1082 **200 preps**

Eco-Friendly Gel Extraction Kit

For research use only

Kit Content

Content	N1081	N1082
Solution N	100 ml	100 ml x2
Wash Buffer PE	15 ml x2	20 ml x3
Eluent buffer	5 ml	10 ml
Spin column	100 each	200 each

Description

The Eco-Friendly system is the only method available that does not utilize Guanidine Salt, a hazardous protein denaturant, in the silica based purification system, which will ensure the safety and health of scientists and the environment.
The purified DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or in vitro transcription/translation without further manipulation.

Features

Safety and environmental protection: No toxic chemicals in the solution, which is safer for people; No toxic chemicals in the waste, which is easy to dispose and good for environment.
High integrity: protect the terminal base pairs of the DNA fragment
Fast:procedure takes 15 minutes.
Highly efficient: 85-100% recoveries
Pure DNA: A260/A280= 1.7-1.9.

Important Notes

• Prior to the initial use of the kit, dilute the Wash Buffer(PE) with ethanol (100%):

	N1081(100preps)	N1082(200preps)
Wash Buffer(PE)	15 ml ×2	20 ml ×2
Ethanol	60 ml ×2	80 ml ×2
Total Volume	75 ml ×2	100 ml ×2

After the ethanol has been added, mark the check box on the bottle to indicate the completed step

Protocol

1. Excise gel slice containing the DNA fragment using a clean scalpel or razor blade. Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a pre-weighed 1.5 ml tube and weigh. Record the weight of the gel slice.

   Note If the purified fragment will be used for cloning reactions, avoid damaging the DNA through UV light exposure. Minimize UV exposure to a few seconds or keep the gel slice on a glass or plastic plate during UV illumination.

2. Add Solution N at a ratio of 300µl of solution per 100mg of agarose gel slices.
3. Incubate the gel mixture at 50-60°C for 10 min or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process. Ensure that the gel is completely dissolved.

   Note • Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is red, add 10 µl of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.

   • High concentration gels (>2% agarose) or large gel slices may take longer than 10 minutes to dissolve.
4. Transfer the dissolved gel mixture to the spin columns assembly and incubate for 2 minute at room temperature.
5. Centrifuge at 12,000 rpm for 1 minute, then discard the flow-through.
6. Wash the column by adding 500 µl of Wash Buffer PE to the spin column. Centrifuge for 1 minute at 12,000rpm, then discard the flow-through.

   Note Wash Buffer PE must previously diluted with 100% ethanol.
7. Wash the column by adding 500 µl of Wash Buffer PE again, previously diluted with100% ethanol, to the spin column. Centrifuge for 1 minute at 12,000rpm, then discard the flow-through.
8. Centrifuge the spin column for an additional 3 min to completely remove residual wash buffer.

   Note This step is essential to avoid residual ethanol in the purified DNA solution. The presence of ethanol in the DNA sample may inhibit downstream enzymatic reactions.
9. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.
10. Carefully transfer the spin column to a clean 1.5ml microcentrifuge tube. Apply 50 µl of Elution Buffer (10 mM Tris-HCl, pH 8.5) directly to the center of the column without touching the membrane with the pipette tip. Incubate at room temperature for 2 minute. Centrifuge for 1 minute at 12,000rpm.

    Note • For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 µl does not significantly reduce the DNA yield. However, elution volumes less than 10 µl are not recommended.

    • If DNA fragment is >10 kb, prewarm Elution Buffer to 65°C before applying to column.

    • If the elution volume is 10 µl and DNA amount is >5 µg, incubate column for 1 min at room temperature before centrifugation.
11. Discard the spin column and store the microcentrifuge tube containing the eluted DNA at 4°C or –20°C.