Gel Extraction Kit

For research use only

Kit Content

Content	DN1071	DN1072	DN1073
Binding Buffer DBD	20 ml	40 ml	80 ml
Wash Buffer DPE	15 ml	30 ml	60 ml
Elution Buffer (10 mM Tris-HCl, pH 8.5)	2.5 ml	5 ml	10 ml
Spin Columns	50 each	100 each	200 each

Description

Gel Extraction Kit is designed to extract and purify DNA fragments of 50bp to 40kb from standard or low-melt agarose gels in either Tris acetate (TAE) or Tris borate (TBE).This membrane-based system, which can bind up to 40µg DNA, allows recovery of isolated DNA fragments in as little as 20 minutes, depending on the number of samples processed and the protocol used. The purified DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or in vitro transcription/translation without further manipulation.

Applications

Fast and efficient extraction of high purity DNA fragments ideal for use in all conventional molecular biology procedures including:

- **Restriction digestion**
- **PCR**
- **DNA Sequencing**
- **In vitro transcription**

Feature.

- **Fast - procedure takes only 15 min.**
- **High Efficient – up to 85% recoveries in the range of 50bp-40kb.**
- **High purity - OD260/280=1.7-1.9. Purified DNA is ready for downstream application such as PCR, restriction digestion.**

Store

Gel Extraction Kit can be stored for up to 12 months at room temperature (15-25°C) or at 4°C for storage periods longer than 12 months. Any precipitate in the buffers can be re-dissolved by incubating at 37°C before use.

Note

- **Prior to the initial use of the kit, dilute the Wash Buffer DPE with ethanol (96-100%):**

	DN1071(50p)	DN1072(100p)	DN1073(200P)
Wash Buffer DPE	15 ml	15 ml x2	15 ml x3
Ethanol	60 ml	60 ml x2	60 ml x3
Total Volume	75 ml	75 ml x2	75 ml x3

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

• Examine the Binding Buffer DBD for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37°C and cooling to 25°C.

• Wear gloves when handling the Binding Buffer DBD as this solution contains irritants.

• If extracted DNA will be used directly for sequencing, freshly prepared electrophoresis buffers should be used both for gel preparation and for gel running

• All centrifugations should be carried out in a table-top microcentrifuge at>12000 g (10,000-14,000 rpm, depending on the rotor type)

Protocol

1. Weigh a 1.5ml microcentrifuge tube for each DNA fragment to be isolated and record the weight.

2. 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.

3. Add Bing Buffer DBD at a ratio of 10µl of solution per 10mg of agarose gel slices.

4. Incubate the gel mixture at 50-60°C for 7-10 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 solutions 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.

5. Transfer the dissolved gel mixture to the Spin Columns assembly and incubate for 2 minute at room temperature.

6 Centrifuge the Spin Columns assembly in a microcentrifuge at ~12,000 rpm for 1 minute, then discard the flow-through.

7. Wash the columns by adding 500 µl of Wash Buffer DPE to the Columns.

Centrifuge the columns assembly for 1 minute at ~12,000 rpm , then discard the flow-through.

Note: Wash Buffer DPE must previously diluted with ethanol(96-100%).

8. Repeat step 7 again.

9. Centrifuge the Columns 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.

10. 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.

11. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl Elution Buffer DTE (prewarm to 60^{0}C) directly to the center of the column without touching the membrane. Incubate at room temperature for 2 min.

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 60°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.

12. Centrifuge for 1 minute at ~12,000 rpm. Discard the columns and store the microcentrifuge tube containing the eluted DNA at –20°C.

Note • Elution buffer can be replaced by deionized water. But the PH should be 8.0-8.5.

• Prewarm Elution Buffer DTE to 60^{0}C can increase the yield of genomic DNA.

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