GeneBio Systems, Inc.™

GB-Clean™ GEL EXTRACTION KIT

Because GB-Clean™ Makes Your Nucleic Acid Pure and Reaction-Ready!

50 Preps Cat. # N1071
100 Preps Cat. # N1072
200 Preps Cat. # N1073

For research use only

Kit Contents

Component	Cat. # N1071 50 Preps	Cat. # N1072 100 Preps	Cat. # N1073 200 Preps
Binding Buffer BG	20mL	2 x N1071	4 x N1071
Wash Buffer PE	15mL	2 x N1071	4 x N1071
Elution Buffer	2.5mL	2 x N1071	4 x N1071
Spin Columns	50	2 x N1071	4 x N1071

Description

GB-Clean™ Gel Extraction Kit is designed to extract and purify DNA fragments of 50bp to 40kb from standard or low-melt agarose gels made in either Tris acetate (TAE) or Tris borate (TBE). This silica membrane-based system, which has a binding capacity 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

Features

- Fast - procedure takes less than 20 minutes.
- Highly Efficient - up to 85% recoveries of DNA in the range of 50bp-40kb.
- Highly purity - OD260/280 of the resulting DNA is typically 1.7-1.9.

Storage

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 the buffer at 37 °C before use.

Notes

• Prior to the initial use of the kit, , dilute the Wash Buffer PE with 60 ml of ethanol (96-100%)(50 preps per kit).

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

• Examine the Binding Buffer BG 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 BG 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 preparing the sample for gel electrophoresis.

• All centrifugation steps should be carried out in a table-top microcentrifuge at >12000 g (on most centrifuges, it is 12,000 rpm or 10,000-14,000 rpm, depending on the rotor type).

Protocol

1. Pre-warm an aliquot of the Elution Buffer to 60 °C to be used at step 12. Volume of Elution Buffer = (# of samples+1) multiplied by 30-100µL.

2. Weigh an empty 1.5mL microcentrifuge tube, and record the weight.

3. 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 the pre-weighed 1.5mL tube and weigh. Calculate and record the weight of the gel slice (weight of tube+gel slice minus wieght of empty tube).

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

4. Add Binding Buffer BG/or BD at a ratio of 20µl of solution per 10mg of agarose gel slices.
   Note: Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is pink/red, add 10µL of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow. Optional: For DNA fragments less than 200 bp, add 1 volume of isopropanol

5. Incubate the gel mixture at 50-60 °C for 7-10 minutes 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 before proceeding.

   Notes: High concentration gels (>2% agarose) or large gel slices may take longer than 10 minutes to dissolve, and require a Binding Buffer BG ratio to gel of 6:1.

6. Transfer the dissolved gel solution to the Spin Columns assembly (column on 2mL collection tube) and incubate for 2 minute at room temperature.

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

8. Wash the columns by adding 500µL of Wash Buffer PE to the Columns . Centrifuge the columns assembly for 1 minute at ~12,000 rpm , then discard the flow-through.
   Note: Wash Buffer PE must previously diluted with ethano l(96-100%).

9. Repeat step 7 once.

10. Centrifuge the Columns for an additional 3 minutes 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.

11. 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.

12. Place the spin column in a clean 1.5mL microcentrifuge tube (not provided), and pipet 30-100µL Elution Buffer TE (prewarmed to 60 °C) directly to the center of the column without touching the membrane. Incubate at room temperature for 2 minutes.

    Notes: For low DNA amounts, the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50µL does not significantly reduce the DNA yield. However, elution volumes less than 10 µl are not recommended.
    • We highly recommend to prewarm Elution Buffer TE to 60 °C, as it can increase the yield of genomic DNA, particularly if DNA fragment is >10 kb.
    • If the elution volume is 10µL and DNA amount is >5µg, incubate column for 1 minute at room temperature before centrifugation.

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

    Notes: Elution buffer can be replaced by deionized water. If water is used, ensure that its pH is 8.0-8.5.

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