Cat.:BR2E301

Universal DNA Purification Kit

V01

Product Description

The Universal DNA Purification Kit uses a nucleic acid purification column to specifically bind DNA. It recovers DNA fragments from TAE and TBE agarose gels, and can also be directly used to purify DNA fragments from crude DNA preparations obtained by other methods such as PCR products or restriction enzyme digestion products, removing impurities including other organic compounds, proteins, inorganic salt ions, oligonucleotide primers, and others. The DNA purified by this kit can be directly used in various applications such as restriction digestion, ligation, cloning, sequencing, and other molecular biology operations.

Components

Components	BR2E301-01 50 T	BR2E301-02 200 T
Buffer GSB	25 mL	100 mL
Buffer PW	15 mL	50 mL
Elution Buffer	15 mL	30 mL
Spin Columns with Collection Tubes	50 sets	200 sets

Storage

The kit is stored dry at room temperature (15–30°C) for 18 months. If precipitation occurs in the solutions, they may be preheated in a 37°C water bath for 10 minutes before use to dissolve the precipitate, which does not affect performance.

Materials

Anhydrous ethanol, 3 M sodium acetate (pH 5.2), and isopropanol (add 1 gel volume of isopropanol when recovering DNA fragments ≤ 100 bp).

Notes

1. For Research Use Only. Not for use in diagnostic procedures.
2. Before the first use of Buffer PW, add 4 volumes of anhydrous ethanol (e.g., add 60 mL anhydrous ethanol to 15 mL Buffer PW; add 200 mL anhydrous ethanol to 50 mL Buffer PW). Store at room temperature.
3. To ensure recovery efficiency, fresh electrophoresis buffer is recommended for electrophoresis.
4. UV irradiation may damage DNA and affect downstream experiments (such as cloning and ligation). Avoid prolonged UV exposure during gel excision and recovery.
5. Buffer GSB contains a pH indicator and appears yellow, indicating pH ≤ 7.5.
6. A larger elution volume results in a higher elution yield. To obtain concentrated DNA, the elution volume may be appropriately reduced, but the minimum volume should be no less than 25 µL. An excessively small volume will reduce DNA elution efficiency and final yield.

Protocol

Note: Add 4 volumes of absolute ethanol to Buffer PW before first use.

I. Recovery of DNA Fragments from Agarose Gels

1. After completion of DNA electrophoresis, excise the gel containing the target DNA fragment under a UV lamp, and remove excess gel as much as possible. Weigh the gel (subtracting the weight of the empty tube); 100 mg of gel is equivalent to 100 µL in volume.
2. Add an equal volume of Buffer GSB and incubate in a water bath at 55–60°C.Gently invert the centrifuge tube 2–3 times during incubation to ensure the gel slice is completely dissolved.After the gel is fully melted, observe the color of the gel solution: If yellow, proceed to subsequent steps; If orange-red or purple, adjust the color to yellow using 10 µL of 3 M sodium acetate (pH 5.2) before proceeding.
3. Allow the melted gel solution to cool down to room temperature, then transfer it to the adsorption column. Let it stand at room temperature for 1 min, centrifuge at 12,000 rpm for 1 min, and discard the filtrate.(The DNA-binding capacity of the adsorption column is weak at high temperatures.)
4. Add 600 µL of Buffer PW (please check if absolute ethanol has been added before first use), centrifuge at 12,000 rpm for 1 min, and discard the filtrate. Repeat this step.
5. Centrifuge at 12,000 rpm for 2 min to completely remove residual Buffer PW.

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6. Place the adsorption column into a clean centrifuge tube, open the lid and leave it at room temperature for 2 min. Add 30–50 μL of Elution Buffer or deionized water (preheating at 60–70°C in advance will improve elution efficiency) to the center of the adsorption column, and let it stand for 1 min. Centrifuge at 12,000 rpm for 2 min to recover the DNA product, and store at −20°C. To increase the product concentration, it is recommended to add the recovered solution back into the adsorption column and repeat this step for a second elution.

II. Recovery of DNA Products from PCR Reaction Mixtures or Restriction Digestion Mixtures

1. Measure the volume of the PCR reaction solution or restriction enzyme digestion solution using a pipette, add 3 volumes of Buffer GSB, and mix thoroughly.After mixing, observe the color of the solution: If yellow, proceed to subsequent steps; If orange-red or purple, adjust the color to yellow using 10 μL of 3 M sodium acetate (pH 5.2) before proceeding. (For recovery of DNA fragments ≤ 100 bp, add 1 volume of isopropanol relative to the PCR or restriction enzyme digestion solution, mix well, then proceed to Step 2.)
2. Transfer the mixed solution to the adsorption column, let it stand at room temperature for 2 min, centrifuge at 12,000 rpm for 1 min, and discard the filtrate.
3. Add 600 μL of Buffer PW (please check whether absolute ethanol has been added before first use), centrifuge at 12,000 rpm for 1 min, and discard the filtrate. Repeat this step.
4. Centrifuge at 12,000 rpm for 2 min to completely remove residual Buffer PW.
5. Place the adsorption column into a clean centrifuge tube, open the lid and let stand for 2 min.
6. Add 30–50 μL of Elution Buffer or deionized water to the center of the adsorption column (preheating at 60–70°C in advance will improve elution efficiency) and let stand for 1 min. Centrifuge at 12,000 rpm for 2 min to recover the DNA product, and store at −20°C. To increase the product concentration, it is recommended to add the resulting solution back into the adsorption column and repeat this step for a second elution.

FAQs

1. Low DNA recovery efficiency: Excessive time spent excising the gel under UV light may cause nucleic acids to be exposed to UV radiation for an extended period, leading to potential alterations in their structure and loss of function. The electrophoresis buffer used for electrophoresis has not been replaced for a long time, resulting in a change in pH, which affects DNA binding.
2. Incomplete melting of the agarose gel may reduce recovery efficiency: Remove as much agarose gel without the target fragment as possible. During the gel-melting process, invert and mix intermittently to promote gel dissolution. Careful observation to ensure complete melting of the gel can improve recovery efficiency.
3. Low elution efficiency: Preheat Elution Buffer to 60–70°C and perform a second elution.
4. Salt contamination: Please elute twice with Buffer PW. Buffer PW can be added along the inner wall of the adsorption column. After addition, cover the tube and invert to mix 2–3 times to help thoroughly wash away residual salts on the tube wall.