TIANGEN®

TIANgel Midi Purification Kit

For DNA purification from agarose gel

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TIANgel Midi Purification Kit

(Spin Column)

Cat. no. 4992443/4992445

Kit Contents

Contents	4992443 50 preps	4992445 200 preps
Buffer BL	30 ml	120 ml
Buffer PN	25 ml	100 ml
Buffer PW	15 ml	50 ml
Buffer EB	15 ml	30 ml
Spin Columns CA2	50	200
Collection Tubes 2 ml	50	200
Handbook	1	1

Storage

TIANgel Midi Purification Kit can be stored dry at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C for 10 min before use.

Introduction

TIANgel Midi Purification Kit is designed to extract and purify DNA from any agarose gel in either TAE or TBE buffer without phenol extraction or ethanol precipitation. The kit combines the convenience of spin-column technology with the selective binding properties of a uniquely designed silica membrane. Special buffers are optimized for efficient recovery of DNA and removal of contaminants. The Spin Column CA2 can purify the fragments ranging from 100 bp to 30 kb with over 80% of recovery efficiency from primers, nucleotides, polymerases, and salts. The spin column could bind 10 μg DNA per column.

TIANgel Midi Purification Kits provide high yields of pure nucleic acids, for direct use in applications such as restriction enzyme digestion, PCR, sequencing, library screening, ligation and transformation, *in vitro* translation, and transfection of robust cells.

Important Notes Before starting

1. Buffer BL can improve the absorption capability and stability of the silica membrane. Check Buffers before use for salt precipitation. Dissolve any precipitate by warming at 37°C for several minutes.
2. Use fresh electrophoresis buffer on electrophoresis.
3. Apply TAE electrophoresis buffer if there is high demand for following experiment.
4. All centrifuge steps are performed in a conventional tabletop microcentrifuge at room temperature (15-30°C).
5. Shorten ultraviolet irradiation time when cutting agarose gel.
6. If the purification efficiency is low, check solution pH after agarose gel is dissolved completely. If pH>7.5, add 10-30 μl of 3 M CH_3COONa (pH 5.2) to the gel solution until the solution pH is adjusted to 5-7.
7. Increase of the absorption and elution time and the volume of Buffer PN could improve recovery efficiency for <100 bp and >10 kb DNA fragment.
8. The purifying efficiency is related to the starting DNA quantity and elution volume. Insufficient starting quantity or elution volume reduces the recovery efficiency significantly.

Protocol

Add ethanol (96-100%) to Buffer PW before use (see bottle label for volume).

1. Column equilibration: add 500 µl Buffer BL to the Spin Column CA2 **(put Spin Column CA2 into a collection tube)**. Centrifuge for 1 min at 12,000 rpm (~13,400 × g) in a table-top microcentrifuge. Discard the flow-through, and put Spin Column CA2 back into the collection tube **(please use freshly treated spin column).**
2. Cut the DNA fragment from agarose gel with a clean, sharp scalpel. Weigh the gel slice in a clean tube.
3. Add equivalent volume of Buffer PN to the gel (If the gel is 0.1 g, it is defaulted to be 100 µl, then add 100 µl Buffer PN). Incubate at 50°C by inverting up and down the tube until the agarose gel dissolves completely. If the agarose gel does not dissolve completely, incubate for longer period or add additional Buffer PN until all the agarose gel dissolved completely **(If the agarose gel is too large, please cut the agarose gel into several pieces in advance).**

   Note: If DNA fragment is <300 bp, it is recommended to add isopropanol which is 1/2 volume of Buffer PN to the agarose gel sample after the gel is completely dissolved. Cool the solution at room temperature (15-30°C) and then add the solution to Spin Column CA2 since silica membrane of the column adsorbs DNA best at room temperature.
4. When the gel dissolved completely and the solution temperature turns to room temperature, transfer the mixture to the Spin Column CA2 (put Spin Column CA2 into a collection tube). Let the column stand for 2 min at room temperature, then centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g) in a table-top microcentrifuge. Discard the flow-through; place the Spin Column CA2 back into the collection tube again.

   Note: The maximum loading volume of the column is 800 µl. For sample volumes greater than 800 µl simply load again.

5. Wash the Spin Column CA2 with 600 µl Buffer PW **(ensure that ethanol (96-100%) has been added)** and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through and place the Spin Column CA2 back into the collection tube.

   Note: If the purified DNA is used for the salt sensitive experiments, such as direct sequencing and blunt-ended ligation, let the column stand for 2-5 min after adding Buffer PW, and then centrifuge.

6. Repeat Step 5.

7. Place the Spin Column CA2 back to the collection tube and centrifuge at 12,000 rpm (~13,400 × g) for 2 min to remove residual Buffer PW. Discard the flow-through, and place column with the cap open for several minutes to air dry the membrane.

   Note: Residual ethanol from Buffer PW will influence the subsequent enzymatic reaction (enzyme digestion, PCR, etc.).

8. Transfer the Spin Column CA2 to a clean 1.5 ml microcentrifuge tube. Add appropriate volume of Buffer EB to the center of the membrane, incubate at room temperature (15-30°C) for 2 min, then centrifuge at 12,000 rpm (~13,400 × g) for 2 min.

   Note: The elution volume should not be less than 30 µl since smaller volume will affect recovery efficiency. The pH value of eluted buffer will affect eluting. If purified DNA is used for sequencing, it is recommended to choose ddH_2O (pH 7.0-8.5) to elute DNA, for pH<7.0 will decrease the elution efficiency. Obtained DNA should be stored at -20°C to prevent degradation. Buffer (10 mM Tris-Cl, pH 8.0) could also be used for DNA elution. For higher yield, pipette the eluate to the center of the membrane again, incubate for 2 min and centrifuge at 12,000 rpm (~13,400 × g) for 2 min.

Determination of DNA Concentration and Purity

The recovered DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. DNA should have a significant absorption peak at OD_{260}. OD_{260} value of 1 is equivalent to about 50 μg/ml double stranded DNA and 40 μg/ml single stranded DNA. The OD_{260}/ OD_{280} ratio should be 1.7-1.9. If ddH_2O is used for the elution instead of the elution buffer, the ratio will be lower, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.