TIANGEN®

TIANgel Maxi Purification Kit

For DNA purification from agarose gel

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TIANgel Maxi Purification Kit

(Spin Column)

Cat. no. 4992895

Kit Contents

Contents	4992895 (50 preps)
Buffer BL	30 ml
Buffer PN	2 × 25 ml
Buffer PW	15 ml
Buffer EB	15 ml
Spin Columns CA3	50
Collection Tubes 2 ml	50
Handbook	1

Storage

TIANgel Maxi DNA 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 Maxi DNA Purification Kit combines the convenience of spin-column technology with the selective binding properties of a uniquely designed silica membrane and special buffer system. The kit is designed to extract and purify DNA from any agarose gel in either TAE or TBE buffer, meantime remove contaminants of protein, other organic compound, salts and primers, etc. The Spin Column CA3 can purify the fragments ranging from 100 bp to 30 kb with over 80% of recovery efficiency . Every spin column could bind up to 20 μg DNA.

TIANgel Maxi DNA Purification Kit provides high yields of pure nucleic acids, for direct use in applications such as restriction enzyme digestion, PCR, sequencing, library screening, ligation, transformation, etc.

Important Notes **Please read the notes before using this kit.**

1. Buffer BL can improve the absorption capability ,uniformity and stability of the silica membrane and eliminate the effect of bad circumstance such as high temperature or humid on silica membrane performance. 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. Shorten ultraviolet irradiation time when cutting agarose gel.
5. If the purification efficiency is low, check solution pH after agarose gel 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 adjusted to 5-7.
6. Increase absorption and elution time could improve recovery efficiency for <100 bp and >10 kb DNA fragment.
7. The purifying efficiency is related to 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). All centrifuge steps are in a conventional tabletop microcentrifuge at room temperature (15-30°C).

1. Column equilibration: add 500 µl Buffer BL to the Spin Column CA3 **(put Spin Column CA3 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 CA3 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: For recovering small fragments <300 bp, you can add 1/2 gel volume of isopropanol after the gel is completely dissolved by Buffer PN to improve the recovery rate. Cooling the solution at room temperature (15-30°C) and then add the solution to Spin Column CA3 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 CA3 **(put Spin Column CA3 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 CA3 back into the collection tube again.

   Note: The maximum loading volume of the column is 800 µl. For sample volumes greater than 800 µl can be loaded in batches.
5. Wash the Spin Column CA3 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 CA3 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 CA3 back to the collection tube and centrifuge at 12,000 rpm (~13,400 × g) for 2 min to remove residual wash buffer. 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 CA3 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 50 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, 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 2 min and centrifuge at 12,000 rpm (~13,400 × g) for 2 min.

Measurement of DNA concentration and purity

The concentration and purity of recovered DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer.

DNA should have a significant absorption peak at OD_{260}. If the OD_{260} value is 1, then it is equivalent to about 50 µg/ml double-strand DNA and 40 µg/ml single-strand DNA.

The OD_{260}/OD_{280} ratio should be 1.7-1.9. If it is not the elution buffer but ddH_2O is used, the ratio will be low, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.