TIANGEN®

TIANgel Purification Kit

Room temperature gel dissolution, fast and high efficient gel recovery

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TIANgel Purification Kit

(Spin Column)

Cat. no. 4992983/4992984

Kit Contents

Contents	4992983 (50 preps)	4992984 (200 preps)
Buffer PE	50 ml	200 ml
Buffer PW	15 ml	50 ml
Buffer TB	15 ml	30 ml
Spin Columns CA5	50	200
Collection Tubes 2 ml	50	200
Gel Cutter	5	20
Handbook	1	1

Storage

TIANgel Purification Kit should 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 Purification Kit uses a silicon matrix material that can efficiently and specifically bind DNA and a unique, and this kit can recover DNA fragments from TAE or TBE agarose gel while removing impurities such as proteins, other organic compounds, inorganic salt ions and oligonucleotide primer, etc. The purified DNA fragment range is 100bp-15kb with over 80% of recovery efficiency. Each spin-column can adsorb 10 μg of DNA.

DNA recovered with this kit can be used for various operations, including enzyme digestion, PCR, sequencing, library screening, ligation, transformation, etc.

Product Features

Fast: The entire operating process is very fast and convenient and can be finished within 10 minutes.

Diversity: This kit is capable of recovering single-strand and double-strand DNA segments, as well as ring-shaped plasmid DNA.

Efficient: The unique spin-column and the specially buffers allow maximum recovery of DNA with a high purity.

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

1. Use fresh buffer when performing electrophoresis to avoid impacts on the electrophoresis and the recovery efficiency.
2. It is better to use TAE buffer if there is high demand for following experiment.
3. All centrifugation steps are performed using a benchtop centrifuge at room temperature.
4. When cutting the gel, the time of ultraviolet radiation shall be kept to the shortest to prevent damage to DNA.
5. Test the pH value after the gel is sufficiently dissolved if the recovery rate is low. If the pH value is greater than 7.5, you may add 10-30 μl 3 M CH_3COONa (pH5.2) into the gel solution containing DNA until the solution pH adjusted to 5-7.
6. The recovery efficiency is related to the initial amount of DNA and the elution volume. The recovery efficiency will be low if the initial DNA amount and the elution volume are small.

Protocol

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

1. Cut the target DNA band from the agarose gel **(remove the extra part as much as possible)** and transfer it into a clean centrifuge tube, and then weigh the gel block.

   Note: When use the Gel Cutter (OSE-GC) for cutting the gel, point the mouth of the Gel Cutter at the target DNA band in the agarose gel and press to cut. After cutting the gel, push the center rod to push the gel block into a clean centrifuge tube. Single cutting and continuous cutting can be carried out according to the width of the gel well.

2. Add Buffer PE of a volume three times to the gel block (if the gel weighs 0.1 g, its volume defaulted to be 100 μl, then add 300 μl of Buffer PE. Use the Gel Cutter to cut the target DNA band from a 1% agarose gel, and the single gel block is about 0.06 g. The weight of the actual gel block is related to the gel concentration and thickness). Dissolve the gel at room temperature (15-30°C) for 5-10 min while inverting the centrifuge tube gently to ensure quick and sufficient dissolution of the gel block. **(Cut the gel block into smaller pieces if it is too big)**.

   Notes: If the fragment is longer than 5 kb or the gel concentration is more than 1.5%, the gel shall be heated for 5-10 min at 50°C. Only transfer the solution into the Spin Columns CA5 when it drops to the room temperature after the gel block is fully dissolved. The adsorption column has a stronger ability to adsorb DNA at room temperature.

3. Add the solution obtained from the step 2 into an Spin Columns CA5 (place the column in the collection tube) and let it rest 2 min at room temperature. Centrifuge at 12,000 rpm (~13,400 × g) for 30-60 sec. Discard the flow-through and place the Spin Columns CA5 back into the collection tube.

   Notes: The maximum loading volume of the column is 800 μl. Add the sample in batches if its volume is larger than 800 μl.

4. Add 600 μl of Buffer PW **(ensure the ethanol has been added)** into the Spin Columns CA5. Centrifuge at 12,000 rpm (~13,400 × g) for 30-60 sec. Discard the flow-through and place the Spin Columns CA5 back into the collection tube.

   Notes: If the DNA recovered is used for salt-sensitive experiments, such as blunt end ligation experiment or direct sequencing, let it rest for 2-5 minutes after adding PW before centrifugation.

5. Repeat step 4.
6. Put Spin Columns CA5 back in the collection tube and centrifuge at 12,000 rpm (~13,400 × g) for 2 min. Put the Spin Columns CA5 at room temperature for a few minutes to dry thoroughly, so that there will be no residual ethanol to affect the next steps.

   Notes: The residual ethanol in the Buffer PW will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.).
7. Place the Spin Columns CA5 into a clean microcentrifuge tube, add appropriate volume of Buffer TB to the center of membrane and incubate at room temperature for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min and then collect the DNA solution.

   Notes: The elution volume shall not be smaller than 30 μl or it will affect the recovery efficiency. The pH value of the buffer has a great impact on the elution efficiency. ddH_2O shall be used as the eluent if the DNA solution is used for sequencing. Make sure the pH value of the eluent is 7.0-8.5, the elution efficiency will be affected if pH<7.0 and the DNA product shall be stored under -20°C to prevent DNA degradation. DNA can also be eluted with 10 mM Tris-Cl (pH8.0). To increase the recovered amount of DNA, pipette the eluate back to column at room temperature for 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 40μg/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.