TIANGEN®

TIANquick Mini Purification Kit

For purification of PCR products,
100 bp to 10 kb

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TIANquick Mini Purification Kit

(Spin Column)

Cat. no. 4992441

Kit Contents

Contents	4992441 50 preps
Buffer BL	30 ml
Buffer PB	30 ml
Buffer PW	15 ml
Buffer EB	15 ml
Spin Columns CB1	50
Collection Tubes 2 ml	50
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Storage

TIANquick Mini 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

TIANquick Mini Purification Kit applies unique silica-membrane technology to purify 100 bp-10 kb DNA fragments with over 80% recovery yield from enzymatic reactions and PCR products, meanwhile remove contaminants of protein, other organic compound, salts and primers, etc. The Spin Column could bind 5 μg DNA per column, especially suits for small sample.

Purified DNA by the kit can be directly used in applications such as restriction enzyme digestion, PCR amplification, sequencing, library screening, ligation, transformation, etc.

Important Notes Before starting

1. This kit is applied in purification of all DNA fragments exist in solution non-selectively. If need to purify specific DNA fragments, meantime remove all other fragments, please choose TIANgel Purification Kit.
2. The volume of Buffer EB adding should be determined by DNA volume before purification: for 1-5 μg DNA, choose Spin Column CB1, add 20-50 μl Buffer EB; for 5-20 μg DNA, choose Spin Column CB2, add 30-100 μl Buffer EB; for 20-30 μg DNA, choose Spin Column CB3, add 50-300 μl Buffer EB.
3. The recovery efficiency is related to the volume of DNA startingand elution volume. Insufficient starting DNA or elution volume reduces the recovery efficiency significantly.
4. Increase absorption and elution time could improve recovery efficiency for <100 bp and >10 kb DNA fragment.
5. Buffer BL can improve the absorption capability and stability of the silica membrane, eliminate the effect of bad circumstance such as high temperature or humid on silica membrane performance. Check Buffer BL before use for salt precipitation. Redissolve any precipitate by warming at 37°C for several minutes.
6. After treated with Buffer BL, use the Spin Column as soon as possible (in one day).

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 CB1 **(put Spin Column CB1 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 then place Spin Column CB1 back into the collection tube **(please use freshly treated spin column)**.
2. Add 5 volumes of Buffer PB to 1 volume of the PCR reaction or enzymatic reaction and mix. It is not necessary to remove mineral oil or kerosene.

   Note: For example, add 250 µl Buffer PB to 50 µl PCR reaction (not including oil).
3. Transfer the mixture to the Spin Column CB1, incubate at room temperature (15-30°C) for 2 min. Centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g) in a table-top microcentrifuge. Discard the flow-through, and then place Spin Column CB1 back into the same collection tube.

   Note: The maximum loading volume of the column is 800 µl. For sample volumes greater than 800 µl simply load again.
4. Add 600 µl Buffer PW **(ensure that ethanol has been added)** to the Spin Column CB1 and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through, and place Spin Column CB1 back in the same collection tube.

   Note: If the purified DNA is used for the subsequent salt sensitive experiments, such as ligation or sequencing experiment, it is suggested to stand for 2-5 min after adding Buffer PW, and then centrifuge.
5. Repeat step 4.
6. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to remove residual Buffer PW. Discard the flow-through, and allow the column to air dry with the cap open for several minutes to dry the membrane.

   Note: Residual ethanol from Buffer PW may inhibit subsequent experiment (enzymatic or PCR reactions).

7. Place the Spin Column CB1 in a clean 1.5 ml microcentrifuge tube. Add 20-50 µl Buffer EB to the center of membrane, incubate for 2 min, and centrifuge for 2 min at 12,000 rpm (~13,400 × g).

   Note: If the volume of eluted buffer is less than 20 µl, it may affect recovery efficiency. The pH value of eluted buffer will have big influence in eluting; distilled water (pH7.0-8.5, adjusted with NaOH) is suggested to elute plasmid DNA, pH<7.0 will decrease elution efficiency. For long-term storage of DNA, eluting in Buffer EB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis. Repeat step 7 to increase plasmid recovery efficiency.

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.