TIANGEN®

TIANquick Maxi Purification Kit

For purification of PCR products, 100 bp to 10 kb

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TIANquick Maxi Purification Kit

(Spin Column)

Cat. no. 4992894

Kit Contents

Contents	4992894 (50 preps)
Buffer BL	30 ml
Buffer PB	60 ml
Buffer PW	15 ml
Buffer EB	15 ml
Spin Columns CB3	50
Collection Tubes (2 ml)	50
Handbook	1

Storage

TIANquick Maxi 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 Maxi DNA 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, meantime remove contaminants of protein, other organic compound, salts and primers, etc. The Spin Column could bind 20 μg DNA per column.

Purified DNA by the kit can be directly used 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. This kit is applied in purification of all DNA fragments exist in solution non-selectively (fragments <50 bp could be removed). If need to purify specific DNA fragments, meantime remove all other fragments, please choose gel purification kits.
2. Adding volume of Buffer EB 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 starting DNA volume and 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 table-top microcentrifuge at room temperature (15-30°C).

1. Column equilibration: add 500 μl Buffer BL to the Spin Column CB3

(put Spin Column CB3 into a collection tube). Centrifuge for 1 min at 12,000 rpm (~13,400 × g). Discard the flow-through, and then place Spin Column CB3 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 paraffin.

   Note: For example, add 500 µl Buffer PB to 100 µl PCR reaction (Excluding paraffin oil volume).

3. Transfer the mixture to the Spin Column CB3 **(put Spin Column CB3 into a collection tube)**, 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 CB3 back into the same collection tube.

   Note: The maximum loading volume of the column is 800 µl. For sample volumes greater than 800 µl can be loaded in batches.

4. Add 600 µl Buffer PW **(ensure that ethanol (96-100%) has been added)** to the Spin Column CB3 and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through, and place Spin Column CB3 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 CB3 in a clean 1.5 ml microcentrifuge tube. Add appropriate volume of 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 50 µl, it may affect recovery efficiency. The pH value of eluted buffer will have big influence in eluting; distilled water (pH 7.0-8.5, 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 DNA recovery efficiency.

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.