

TIANquick Midi Purification Kit

For purification of PCR products,
100 bp to 10 kb

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TIANquick Midi Purification Kit

(Spin Column)

Cat. no. 4992439/4992440

Kit Contents

Contents	4992439 50 preps	4992440 200 preps
Buffer BL	30 ml	120 ml
Buffer PB	30 ml	120 ml
Buffer PW	15 ml	50 ml
Buffer EB	15 ml	30 ml
Spin Columns CB2	50	200
Collection Tubes 2 ml	50	200
Handbook	1	1

Storage

TIANquick 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

TIANquick Midi 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 10 μ g DNA per column.

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 it is needed 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 μ g DNA, choose Spin Column CB1, add 20-50 μ l Buffer EB; for 5-20 μ g DNA, choose Spin Column CB2, add 30-100 μ l Buffer EB; for 20-30 μ g DNA, choose Spin Column CB3, add 50-300 μ l 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. All centrifuge steps are performed in a conventional table-top microcentrifuge at room temperature (15-30°C).
5. Increase of absorption and elution time could improve recovery efficiency for <100 bp and >10kb DNA fragment.
6. 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.

7. 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).

1. Column equilibration: add 500 μ l Buffer BL to the Spin Column CB2 (**put Spin Column CB2 into a collection tube**). Centrifuge for 1 min at 12,000 rpm (\sim 13,400 \times g). Discard the flow-through, and then place Spin Column CB2 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 μ l Buffer PB to 50 μ l PCR reaction (not including oil).

3. Transfer the mixture to the Spin Column CB2, incubate at room temperature (15-30°C) for 2 min. Centrifuge for 30-60 sec at 12,000 rpm (\sim 13,400 \times g) in a table-top microcentrifuge. Discard the flow-through, and then place Spin Column CB2 back into the same collection tube.

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

4. Add 600 μ l Buffer PW (**ensure that ethanol (96-100%) has been added**) to the Spin Column CB2 and centrifuge for 30-60 sec at 12,000 rpm (\sim 13,400 \times g). Discard the flow-through, and place Spin Column CB2 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 CB2 in a clean 1.5 ml microcentrifuge tube. Add 30-50 μ l 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 30 μ l, 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, for 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 OD₂₆₀. OD₂₆₀ value of 1 is equivalent to about 50 μ g/ml double stranded DNA and 40 μ g/ml single stranded DNA. The OD₂₆₀/OD₂₈₀ ratio should be 1.7-1.9. If ddH₂O 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.