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# TIANquick N96 Purification Kit

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# TIANquick N96 Purification Kit

**(N96 Plate)**

**Cat. no. 4992871/4992872**

## Kit Contents

| Contents | 4992871<br>4 plates | 4992872<br>24 plates |
|---|---|---|
| Buffer BL | 240 ml | 3 × 500 ml |
| Buffer PB | 240 ml | 3 × 500 ml |
| Buffer PW | 3 × 50 ml | 2 × 500 ml |
| Buffer TB | 60 ml | 240 ml |
| N96 Plate CB2 (H) | 4 | 24 |
| N96 Well Plate | 8 | 48 |
| 250 ml Bottle | -- | 1 |
| Plate Cover | 4 | 24 |
| Handbook | 1 | 1 |

## Storage

The kit can be stored under dry conditions at room temperature (15-25°C) for 12 months. For longer storage, please store at 2-8°C. If the solution produces precipitation under the temperature of 2-8°C, place at room temperature for a period of time before use. If necessary, it can be placed in a 37°C water bath for 10 min to dissolve the precipitation.

## Introduction

The kit uses a unique adsorption column to purify DNA fragments in reaction solutions such as enzyme digestion and PCR. It can remove contaminants such as protein, other organic compound, salts and primers. The fragments purified range from 100 bp to 30 kb with over 80% of recovery efficiency. The maximum amount of DNA that can be adsorbed per well is 5 μg.

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 whole operation process only takes a few tens of minutes, saving time to the greatest extent.

**Efficient:** The unique adsorption plate and the specially prepared buffers allow maximum recovery of DNA with a high purity.

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

1. This kit is suitable for non-selective recovery of all DNA fragments in the solution (small fragments below 30 bp can be removed). If necessary to recover specific fragments while removing other fragments of different sizes, please select the gel purification kit.
2. The recovery yield is related to the initial amount of DNA and elution volume. The lower the initial amount of DNA, the smaller the elution volume, and the lower the recovery yield. The elution volume should preferably not be less than 60 μl per well.
3. Before each use, mix 200 ml ethanol (96-100%) with 50 ml Buffer PW.
4. Before use, check whether the Buffer BL is turbid. If there is turbid phenomenon, incubate in a 37°C water bath for a few minutes.
5. Treating the N96 Plate CB2 with the Buffer BL before use can active the silicon matrix membrane and improve the yield.
6. The N96 Plate CB2 treated with Buffer BL is best used on the same day, and the effect will be affected if it is placed for too long.

## Protocol: Users can choose the centrifugal method or negative pressure method.

**The centrifugal protocol**

1. Plate Balance Steps: The N96 Plate CB2 was stacked on the N96 Well Plate, 500 µl of Buffer BL was added into each well, and centrifuged at 3,600 rpm (~ 2,130 × g) for 3 min, then the waste liquid was discarded, and N96 Plate CB2 was put back on the N96 Well Plate. **(Please use the N96 Plate CB2 treated on the same day)** .
2. Estimate the volume of PCR reaction solution or enzyme digestion reaction solution, and add 3 times the volume of Buffer PB to it.

   **For example: If the PCR reaction system is 100 µl (excluding paraffin oil volume), 300 µl of Buffer PB is added. If the volume of the mixed liquid exceeds the volume of the PCR tube, the PCR reaction liquid or enzyme digestion reaction liquid can be transferred to a N96 Well Plate, mixed with the Buffer PB, and then downstream experiments can be carried out.**
3. After sufficient mixing, all the above mixed liquid is transferred to the N96 Plate CB2 balanced in the first step. Place at room temperature for 2 min, centrifuge at 3,600 rpm (~ 2,130 × g) for 5 min, discard the waste liquid, and put the N96 Plate CB2 back into the same N96 Well Plate.

   **Notes: The maximum loading volume of each N96 Plate CB2 column is 700 µl. If the sample volume is more than 700 µl, it can be added into centrifugation in batches.**
4. Add 700 µl of Buffer PW to each well of N96 Plate CB2 **(please check whether ethanol has been added before use)**, centrifuge at 3,600 rpm (~ 2,130 × g) for 3 min, discard the waste liquid, and put the N96 Plate CB2 back into the same N96 Well Plate.
5. Repeat Step 4.
6. 3, 600 rpm (~ 2,130 × g) centrifuge for 10 min in order to remove the residual Buffer PW .

   **Notes: The residue of ethanol will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.) .**
7. The N96 Plate CB2 was placed in a new N96 Well Plate, and 80-100 µl $ddH_2O$ (pH ≥ 7.5) or Buffer TB was added to each well of N96 Plate CB2, and the DNA solution is collected by centrifugation at 3,600 rpm (~ 2,130 × g) for 10 min at room temperature for 5 min.

**Notes: Usually 80 µl Buffer TB can elute 50 µl DNA product on average. In order to improve the yield, the eluent volume can be increased. In addition, if eluting with $ddH_2O$, the pH value should be kept in the range of 7.0-8.5.**

## Vacuum protocol

1. Connect the negative pressure vacuum device correctly and place the N96 Plate CB2 on the negative pressure vacuum device. Add 200 µl of Buffer BL to each well of N96 Plate CB2, turn on and adjust the negative pressure vacuum, and absorb all the solution in the plate.
2. Adding 3 times the volume of Buffer PB to PCR, enzyme digestion, enzyme labeling or sequencing reaction solution; After mixing evenly, transfer to balanced N96 Plate CB2, place at room temperature for 2 min, turn on and adjust negative pressure to absorb all the solution in the plate.
3. 200 µl of Buffer PW was added to each well of N96 Plate CB2 to absorb all that solution in the plate.
4. Repeat Step 3.
5. The N96 Plate CB2 was sucked at the maximum negative pressure for 10 min.

   **Notes: The residue of ethanol will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.) .**
6. The N96 Plate CB2 is slapped 6 times on the absorbent paper with the guide pipe facing down.
7. The N96 Plate CB2 is placed on the N96 Well Plate, and 80-100 µl $ddH_2O$ (pH ≥ 7.5) or Buffer TB is added, stand at room temperature for 5 min. Centrifuge at 3,600 rpm for 10 min and collect the DNA solution.
8. The N96 Well Plate was covered with a new plate cover, and store the DNA solution at -20°C for standby.

## Determination of DNA concentration and purity

The concentration and purity of DNA 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 ratio of $OD_{260}/OD_{280}$ should be 1.7-1.9. If eluted with $ddH_2O$ instead of Buffer TB, the ratio will be low, but it does not mean low purity, because pH value and ion presence will affect light absorption value.