TIANGEN®

# TIANprep Rapid Mini Plasmid Kit

For fast purification of plasmid DNA of molecular biology grade

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# TIANprep Rapid Mini Plasmid Kit

**(Spin Column)**

**Cat.no. 4992191/4992192**

## Kit Contents

| Contents | 4992191<br>50 preps | 4992192<br>200 preps |
|---|---|---|
| RNase A (10 mg/ml) | 150 µl | 600 µl |
| Buffer P1 | 15 ml | 60 ml |
| Buffer P2 | 15 ml | 60 ml |
| Buffer P5 | 20 ml | 80 ml |
| Buffer PWT | 15 ml | 50 ml |
| Buffer TB | 15 ml | 30 ml |
| TIANRed | 75 µl | 300 µl |
| Spin Columns CP3 | 50 | 200 |
| Collection Tubes 2 ml | 50 | 200 |
| Handbook | 1 | 1 |

## Storage

TIANprep Rapid Mini Plasmid 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 to 37°C before use. RNase A (10 mg/ml) can be stored for 15 months at room temperature (15-30°C). After addition of RNase A and TIANRed, Buffer P1 is stable for 6 months at 2-8°C.

## Introduction

The method that used in TIANprep Rapid Mini Plasmid Kit is optimized from traditional alkaline lysis technology, by which high-quality plasmid DNA could be purified within 8 minutes. The new lysis buffer allows the adsorption of DNA onto silica membrane in the presence of high salt. The material that used to make the silica membrane is unique, highly-efficient and highly-specified. This protocol is designed for purification of DNA from 1-4 ml overnight cultures of E. *coli*.

Plasmid DNA prepared by TIANprep Rapid Mini Plasmid Kit is suitable for a variety of routine applications including restriction enzyme digestion, sequencing, library screening, ligation and trans-formation, in vitro translation, and transfection of robust cells.

## Yield

| Plasmid Type | Bacterial Cells Volume | Plasmid Yield | Plasmid |
|---|---|---|---|
| Low Copy | 1-4 ml | 3-10 μg | pBR322, pACYC, pSC101, SuperCos, pWE15 |
| High Copy | 1-4 ml | 6-24 μg | pTZ, pUC, pBS, pGM-T |

## Important Notes

1. Add the provided RNase A and TIANRed solutions to Buffer P1 before use, mix, and store at 2-8°C.
2. Check Buffer P2 and P5 before use for salt precipitation. Redissolve any precipitate by warming at 37°C.
3. Avoid contacting Buffer P2 and P5 directly, and immediately close the lid after use.
4. All centrifugation steps are carried out at 12,000 rpm (~13,400× g) in table-tap microcentrifuge at room temperature (15-30°C).
5. The obtained plasmid amount is influenced by bacteria culture density and plasmid copy number as well.
6. TIANRed user guide: TIANRed is an indicator, which is harmless and used to make sure that the whole experimental process works well. TIANRed should be mixed with Buffer P1 in the ratio of 1:200 and the color of the mixed solution should be clear red. Add the mixed solution to cell

culture and the solution would turn turbid red. After that, add Buffer P2 to the turbid solution, the solution would turn clear purple which means a complete lysis. Add Buffer P5 to the purple solution and it would turn clear yellow, which indicate that the neutralization reaction has been done.

## Protocol

**Please add ethanol (96-100%) to Buffer PWT before use (check bottle label for volume)**

1. Harvest 1-4 ml bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400× g) in a conventional, table-top microcentrifuge for 1 min at room temperature (15-30°C), then remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.
2. Resuspend pelleted bacterial cells in 150 µl Buffer P1 by pipetting or vortex **(Ensure that RNase A and TIANRed have been added to Buffer P1).**

   **Note: Cell clumps indicate incomplete lysis, which will result in lower yield and purity.**

   **Addition of TIANRed will not have negative impact on following PCR, enzyme digestion and sequencing. TIANRed should be mixed with Buffer P1 in the ratio of 1:200 and the color of the mixed solution should be clear red. Add the mixed solution to cell culture and the solution would turn turbid red.**
3. Add 150 µl Buffer P2 and mix gently by inverting the tube 6-8 times.

   **Note: Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. If not clear, probably due to incomplete lysis, please reduce the cells.**

   **Since TIANRed is applied, after the addition and mix of Buffer P2, the solution should turn clear purple. If there is still some red turbidity can be seen in the tube, keep inverting the tube until the color of solution turns completely clear purple.**
4. Add 350 µl Buffer P5 and mix immediately and quickly by inverting 12-20 times. The solution should become cloudy. Centrifuge for 2 min at

12,000 rpm (~13,400× g) in a table-top microcentrifuge.

**Note: To avoid localized precipitation, mix the solution quickly, immediately after addition of Buffer P5. The solution should be centrifuged again if there is still a lot of white precipitate can be seen in the supernatant. Since TIANRed is applied, after the addition and mix of Buffer P5, the solution should turn clear yellow. If there is still some purple liquid can be seen in the tube, keep inverting the tube until the color of solution turns completely clear yellow.**

5. Transfer the supernatant from step 4 to the Spin Column CP3 **(put in a Collection Tube)** by pipetting. Centrifuge for 30 s at 12,000 rpm (~13,400× g). Discard the flow-through and set the Spin Column CP3 back into the Collection Tube.
6. Wash the Spin Column CP3 by adding 300 µl Buffer PWT **(ensure the ethanol (96-100%) has been added to Buffer PWT)** and centrifuging for 30 s at 12,000 rpm (~13,400× g). Discard the flow-through, and put the Spin Colum CP3 back into the Collection Tube.
7. Centrifuge for an additional 1 min at 12,000 rpm (~13,400× g) to remove residual wash buffer.
8. Place the Spin Column CP3 in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50-100 µl Buffer TB to the center of the Spin Column CP3, centrifuge for 30 s at 12,000 rpm (~13,400 × g).

   **Note: The volume of elution buffer should not be less than 50 µl, otherwise it may affect recovery efficiency. The pH value of elution buffer will have a great effect on eluting.**