TIANGEN®

HighPure Maxi Plasmid Kit

For purification of high pure plasmid DNA with high yield

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HighPure Maxi Plasmid Kit

Cat. no. 4992437

Kit Contents

Contents	4992437 (10 preps)
RNase A (100 mg/ml)	500 μl
Buffer P1	100 ml
Buffer P2	100 ml
Buffer P4	100 ml
Buffer TB	30 ml
TIANRed	500 μl
Filtration CS1	10
Handbook	1

Storage

HighPure Maxi Plasmid Kit can be stored 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 (100 mg/ml) can be stored for 15 months at room temperature (15-30°C). After adding RNase A, Buffer P1 is stable for 6 months at 2-8°C.

Introduction

HighPure Maxi Plasmid Kit adopts specially designed buffer system that is used in conjunction with isopropanol precipitation to achieve fast and efficient purification of high pure plasmid DNA. Plasmid DNA prepared by HighPure Maxi Plasmid Kit is suitable for a variety of routine applications including restriction enzyme digestion, sequencing, library screening, ligation and transformation, in vitro translation, and transfection to cells.

Recommended bacterial culture volume: 500-1500 μg plasmid from 100 ml bacterial culture for high-copy plasmid; and 200-400 μg plasmid from 200 ml bacterial culture for low-copy plasmid.

Important notes Please read before use.

1. Add the provided RNase A solution to Buffer P1 (**use 1 vial RNase A per bottle Buffer P1**), mix, and store at 2-8°C.
2. Check Buffer P2 and P4 before use for salt precipitation. If necessary, dissolve the buffer by warming at 37°C for several minutes.
3. Prepare about 60 ml of 5 M NaCl buffer.
4. Avoid direct contact of Buffer P2 and P4, immediately close the lid after use.
5. Draw out the plunger from the Filtration CS1 slowly to avoid membrane loose.
6. The amount of extracted plasmid is related to cells concentration and plasmid copy. If working with low copy vectors or large plasmid (>10 kb), it may be beneficial to increase culture volume and to increase Buffer P1, P2, and P4 in proportion. Warm the Buffer TB at 65-70°C before use.
7. **TIANRed user guide:** TIANRed is an indicator, which is harmless and used to make sure that the whole experimental process works well. TIANRed is optional, researchers can determine whether to use according to their experience and experiment purpose. **If the purified plasmid is to be used in transfection experiment, TIANRed is not recommended.** 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 bacterial cells 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 P4 to the purple solution and it would turn clear yellow, which indicate that the neutralization reaction has been done.

Protocol:

1. Harvest 100 ml (**for low copy plasmid, please harvest 200 ml**) overnight cultured bacterial cells by centrifuging at 10,000 rpm (~11,500 × g) for 3 min at room temperature (15-30°C).

   Note: For large volume of bacterial cells, please harvest to one tube by several centrifugation steps.

2. Remove all traces of supernatant, and invert the open centrifuge tube on filtration paper until all medium has been drained.

3. Resuspend pelleted bacterial cells in 10 ml Buffer P1 **(Ensure that RNase A has been added)**. The bacteria should be resuspended completely by vortex or pipetting up and down until no cell clumps remain.

   Note: No cell clumps should be visible after re-suspension of the pellet, otherwise incomplete lysis will 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 bacterial cells and the color of the solution would turn turbid red. If the purified plasmid is to be used in transfection experiment, TIANRed is not recommended.

4. Add 10 ml Buffer P2 and mix immediately and thoroughly by inverting the tube 6-8 times, and incubate at room temperature for 5 min.

   Note: Mix 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, reduce bacterial pellets.

   If TIANRed has been added to Buffer P1, the cell suspension will turn purple after addition of Buffer P2. If there is still some red turbidity can be seen in the tube, keep inverting the tube until the color of the solution turns completely clear purple.

5. Add 10 ml Buffer P4, and mix immediately by gently inverting 6-8 times, until the whole solution become white cloudy. Incubate at room temperature for 10 min. Centrifuge for 5-10 min at 10,000 rpm (~11,500 × g), the white material should be in the bottom of the centrifuge tube **(if necessary, prolong centrifugation time properly)**. Transfer the supernatant into a Filtration CS1 **(avoid transferring large white precipitates into the Filtration CS1, which will clog the filtration membrane)**. Gently insert the plunger into the Filtration CS1 and filter the cell lysate into a new 50 ml tube (not supplied in the kit).

   Note: To avoid localized precipitation, mix the lysate thoroughly and immediately after addition of Buffer P4. It will not affect filtration if there is small white precipitate in the supernatant that transferred to Filtration CS1. If using more than 100 ml bacterial culture, prolong centrifugal time to 20-30 min.

   Since TIANRed is applied, after the addition and mix of Buffer P4, 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.

6. Add 0.35 volume isopropanol to the filtrate from step 5 and 5 M NaCl of 0.5 volume of isopropanol to the cleared filtrate, mix completely by reverting upside and down.

   Note: it is a normal phenomenon if no visible precipitate appears in this step.

7. Centrifuge for 30 min at 10,000 rpm (~11,500 × g) at 4°C. Discard the supernatant gently and invert the open centrifuge tube on filtration paper.

8. Wash the precipitate by adding 6 ml 70% ethanol. Centrifuge at 10,000 rpm (~11,500 × g) for 10 min at 4°C. Decant all the supernatant without disturbing the precipitate, and invert the open centrifuge tube on filtration paper.

9. Repeat step 8.
10. Incubate the tube at room temperature with lid open for 10-20 min to air dry the ethanol. Redissolve the precipitate with 1-1.5 ml Buffer TB.

    Note: If the volume of eluted buffer is less than 1 ml, it may affect recovery efficiency. The eluted DNA should be stored at -20°C to avoid degradation. The pH value of elution buffer will have great influence in eluting. If using distilled water, pH should be controlled at 7.0-8.5, below 7.0 will affect elution efficiency.