HiPure Plasmid Plus 96 Kit

Introduction

The HiPure Plasmid Plus 96 Kit enables up to 96 minipreps to be performed simultaneously in less than 45 minutes on the Vacuum Manifold (Qiavac 96). This kit provides a fast, simple, and cost-effective plasmid DNA high-throughput method for routine molecular biology laboratory applications. HiPure Kits use silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Plasmid DNA purified with Kits is immediately ready for use. Phenol extraction and ethanol precipitation are not required, and high quality plasmid DNA is eluted in a small volume of Tris buffer or water.

Kit Contents

Product Number	P100601	P100602	P100603
Purification Times	1 x 96 Preps	4 x 96 Preps	20 x 96 Preps
RNase A	5 mg	20 mg	100 mg
Buffer P1	30 ml	120 ml	600 ml
Buffer P2	30 ml	120 ml	600 ml
Buffer P3	40 ml	180 ml	800 ml
Buffer PW1	100 ml	500 ml	2 x 1000 ml
Buffer PW2*	50 ml	2 x 100 ml	4 x 200 ml
Elution Buffer	15 ml	60 ml	300 ml
Lysate Clear Plate	1	4	20
HiPure DNA Plate	1	4	20
1.6 ml Collection Plate	1	4	20
0.8ml Collection Plate	1	4	20

Storage and Stability

The Kit components can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions. If any precipitates form in the buffers,warm at 37℃ to dissolve. After addition of RNase A, Buffer P1 is stable for 6 months when stored at 2–8°C.

Materials and Equipment to be Supplied by User

- Dilute Buffer PW2 with 200ml (1x96 Preps), 2x400ml (4x96 Preps) or 4x800ml (20x96 Preps) 100% ethanol and store at room temperature
- Add the vial of RNase A to the bottle of Buffer P1 and store at 2-8°C
- Heat Elution Buffer to 70°C if plasmid DNA is >10kb

Protocol (Vacuum)

1. **Fill each well of a 96-well S-Block with 1.3 ml of growth medium containing the appropriate selective agent.** Inoculate each well from a single bacterial colony.
2. **Incubate the cultures for 20–24 h at 37°C with vigorous shaking.** The wells in the block may be protected against spill-over by covering the block with a plastic lid or adhesive tape. If nonporous tape is used, use a needle to pierce 2–3 holes in the tape above each well for aeration.
3. **Harvest the bacterial cells in the plate by centrifugation for 5 min at 2100 x *g* in a centrifuge with a rotor for microtiter plates, preferably at 4–10°C.**
   The block should be covered with adhesive tape during centrifugation. Remove media by inverting the block. To remove the media, peel off the tape and quickly invert the block over a waste container. Tap the inverted block firmly on a paper towel to remove any remaining droplets of medium.
4. **Resuspend pelleted bacterial cells in 250µl Buffer P1.**
   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
5. **Add 250 µl Buffer P2 to each sample.** Dry the top of the plate with a paper towel, seal the block with the tape provided, gently invert the block 5–8 times to mix, and incubate at room temperature for 5 min.
   It is important to mix gently by inverting the block. Do not shake vigorously, as this will result in shearing of genomic DNA. If necessary, continue inverting the block until the solution becomes viscous and slightly clear.
6. **Remove the tape from the block. Add 350 µl Buffer P3 to each sample, dry the top of the plate with a paper towel, and seal the block with a new tape sheet.** Gently invert the block 4–8 times. To avoid localized precipitation, mix the samples gently but thoroughly, immediately after addition of Buffer P3. The solutions should become cloudy.

7. **Remove the tape from the block. Pipet the lysates from step 5 (850 µl per well) into the wells of the Lysate Clear plate.** Unused wells of the Lysate Clear plate should be sealed with tape. Apply the vacuum until all samples have passed through.The optimal flow rate is approximately 1–2 drops per second.
   Spin Protocol(Step 7-11): Place the Lysate Clear Plate or HiPure DNA Plate onto 1.6ml Collection Plate, and centrifuge at 1800~3000 x g for 3min.

8. Switch off the vacuum and ventilate the HiPure DNA Plate slowly. Discard the Lysate Clear plate.

9. **Transfer the HiPure DNA plate containing the cleared lysates to the top plate of the manifold. Seal any unused wells of the DNA plate with tape. Replace the plate holder in the base with waste tray. Place the top plate squarely over the base, making sure that the DNA plate is seated securely.** Apply the vacuum. The flow through is collected in the waste tray.

10. **Recommended: Switch off the vacuum, and wash HiPure DNA plate by adding 0.9 ml Buffer PW1 to each well and applying the vacuum.**

11. **Switch off the vacuum. Wash the HiPure DNA Plate by adding 0.9 ml of Buffer PW2 to each well and applying the vacuum.**

12. **Switch off the vacuum. Wash the HiPure DNA Plate by adding 0.5 ml of absolute ethanol to each well and applying the vacuum.**

13. After absolute ethanol has been drawn through all wells, apply maximum vacuum for an additional 10 min to dry the membrane.
    This step removes residual absolute ethanol from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off the vacuum regulator or leakage valves if they are used), allowing maximum airflow to go through the wells.

    Optional:then dry the HiPure DNA Plate at 55oC for 5 min.

14. Switch off the vacuum, and ventilate the HiPure DNA Plate slowly. Lift the top plate from the base, vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the DNA plate with clean absorbent paper.
    This step removes residual Buffer PW2, which may be present around the outlet nozzles and collars of the HiPure plate. Residual ethanol from Buffer PW2 may inhibit subsequent.
    If necessary, incubate at 56oC for 10 min to completely dry the matrix.

15. For elution into a 0.8ml Elute Plate: Replace waste tray with 0.8ml Elute Plate. Place a 96-well microplate directly on the rack. Place the top plate back on the base, making sure that the HiPure DNA plate is positioned securely.

16. **To elute DNA, add 80-100µl of Elution Buffer (10 mM Tris·Cl, pH 8.5) or water to the center of each well of the HiPure DNA plate, let stand for 2 min, and apply maximum vacuum for 5 min.** Switch off vacuum and ventilate QIAvac 96 slowly. For increased DNA concentration, an elution volume of 75 µl can be used.

    Spin Protocol(Step 15): Place HiPure DNA Plate onto 0.5ml Collection Plate, and centrifuge at 3000~5000 x g for 3 min.
    Important: Ensure that the elution buffer is dispensed directly onto the center of HiPure membrane for complete elution of bound DNA. Please note that the average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume.

Troubleshooting Guide

1. Low DNA yields

- **Buffer PW2 did not contain ethanol:** Ethanol must be added to Buffer PW2 before used.
- **Poor cell lysis:** Cells may not have been dispersed adequately prior to the addition of Buffer P2. Vortex to completely resuspend the cells.
- **Column matrix lost binding capacity during storage:** Follow the Optional Protocol for Column Equilibration prior to transferring the cleared lysate to the Column. Add 100µL 3M NaOH to the column prior to loading the sample. Centrifuge at 3000 rpm for 3min. Discard the filtrate.

2. Plasmid DNA floats out of well while loading agarose gel

Ethanol was not completely removed from column following wash steps, centrifuge column as instructed to dry the column before elution.

3. High molecular weight DNA contamination of product

Do not vortex or mix aggressively after adding Buffer P2. Overgrown cultures contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours.

4. Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A260/A280 ratio is high or low)

- **Plasmid DNA is contaminated with RNA:** RNase A treatment is insufficient Confirm that the RNase A Solution was added to Buffer P1 prior to first use. The RNase A solution may degrade due to high temperatures (>65 °C) or prolonged storage (> 6 months at room temperature).
- **Background reading is high due to silica fine particulates:** Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.