HiPure Plasmid EF 96 Kit

Introduction

The HiPure Minipreps system provides a fast, simple, and cost-effective plasmid DNA miniprep method for routine molecular biology laboratory applications. HiPure Miniprep Kits use silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Plasmid DNA purified with Mini 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	P115701	P115702	P115703
Purification Times	1 x 96 Preps	4 x 96 Preps	20 x 96 Preps
RNase A	10 mg	20 mg	100 mg
Buffer P1	30 ml	120 ml	550 ml
Buffer P2	30 ml	120 ml	550 ml
Buffer LEN3	15 ml	60 ml	300 ml
Buffer LN4	80 ml	270 ml	2 x 700 ml
Buffer LN5	40 ml	220 ml	1100 ml
Buffer PW1	40 ml	220 ml	1100 ml
Buffer PW2*	25 ml	100 ml	2 x 200 ml
Elution Buffer	30 ml	60 ml	250 ml
Lysate Clear Plate	1	4	20
HiPure DNA Plate	1	4	20
1.6 ml Collection Plate	2	8	40
0.5ml 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.

Materials and Equipment to be Supplied by User

- Dilute Buffer PW2 with 100ml (1x96 Preps), 400ml (4x96 Preps) or 2x800ml (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

1. **Fill each well of a 96-well S-Block with 1.3~1.5 ml of growth medium containing the appropriate selective agent. Inoculate each well from a single bacterial colony. 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.

2. **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.** Remove media by inverting the block. 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.

3. **Add 260µl Buffer P1 and thoroughly resuspend cell pellets by vortexing, shaking or pipette mixing.**
   Ensure that RNase A has been added to Buffer P1. Pipette mixing and shaking are most often used for automated processes. Pipette mix at least 20 times (if possible, pipette mix 5 times at 4 different locations in the well) for an even resuspension. Alternatively, shake 4 minutes at 600 to 1,200 RPM on a shaker. Shaking speeds will vary, depending on the orbit of the shaker. Vortexing will take 2 to 3 minutes on a high setting. The cell pellet should be completely resuspended so that the mixture appears homogeneous and has no cell clumps.

4. **Add 260µl Buffer P2 and allow the samples to lyse for 5 minutes.**
   Shake 5 minutes at 300-600 RPM. Alternatively, gently pipette mix two times, and then allow the samples to sit for 5 minutes for a complete lysis. Vigorous pipette mixing is not recommended, as BACs can be easily sheared. Do not allow samples to lyse for longer than 10 minutes. If a white precipitate is seen in the P2 solution prior to addition, warm the bottle in a 37°C water bath or under hot running water, shaking periodically, until the precipitate dissolves. It is recommended that gloves are worn when handling P2 , as it is a basic solution.

5. **Add 130µl Buffer N3 and shake 10 minutes on an orbital or linear shaker to neutralize samples.**
   Addition of Buffer N3 neutralizes the solution and precipitates proteins and cellular debris, creating a white flocculent. BACs must be shaken to help release the large template from the flocculent; the same orbital shaker can be used for overnight culture (room temperature or 37°C are both fine). It is recommended that gloves are worn when handling Buffer N3 .

6. Plate the Lysate Clear Plate into 1.6ml Collection Tube.

7. **Pipet the lysates from step 5 into the wells of the Lysate Clear plate.** Centrifuge at 3000 x g from 3min.

8. **Discard the lysate Clear Plate and add 650µl Buffer LEN4 to the lysate.** Pipit up and down 5 times and Transfer the mixture into the HiPure DNA Plate.

9. Plate the HiPure DNA Plate into 1.6ml Collection Tube.

10. **Pipet 650µl of the mixture from step 8 into HiPure DNA plate placed in a 1.6ml collection plate (supplied).** Centrifuge for 3 min at >3,000 x *g* and discard the flow-through. Reuse collection plate.

11. Repeat step 10 with remaining sample. Discard flow-through and Reuse the collection Plate.

12. **Add 500µl Buffer LN5 to each well of plate and centrifuge for 3 min at >3,000 x *g* .** Discard the flow-through and reuse the collection plate.

13. **Add 500µl Buffer PW1 to each well of the plate and centrifuge for 3 min at >3,000 x *g*.**

14. **Add 700µl Buffer PW2 to each well of the plate and centrifuge for 3 min at >3,000 x *g*.**

15. Discard the flow through and reuse the collection plate. Centrifuge at >4,000 x g for 10 min.

16. Allow to air dry for 10 min at room temperature.

17. **Transfer the plate onto a 0.5 ml Collection Plate, and pipet 100µl Elution Buffer directly onto the membrane.** Incubate for 2 min at room temperature, and then centrifuge for 3 min at >3,000 x *g* to elute.
    A second elution step with a further 100~150µl Buffer AE increases yields by up to 20%. For samples containing less than 1 µg DNA, elution in 80 µl Buffer AE or water is recommended.

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 13000 rpm for 30 seconds. 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.