

HiPure Plasmid EF Midi Plus Kit

Introduction

This product designs with unique buffer systems and optimized silica-membrane purification technology. It is suitable for isolating high-concentration, low-endotoxin plasmid DNA from 25~50ml bacterial cultures. The purified plasmid DNA yield is up to 500µg, with an endotoxin level of <1 EU/µg and a concentration of up to 3 µg/µl. It can be used directly for cell transfection. Following further purification using the Additional Protocol, the endotoxin level can be reduced to ≤0.1 EU/µg, making the DNA suitable for animal injection applications.

Kit Contents

Product Number	P123101	P123102	P123101B	P123102B
Purification Times	4 Preps	50 Preps	4 Preps	50 Preps
RNase A	5 mg	30 mg	5 mg	30 mg
Buffer E1	30 ml	170 ml	30 ml	170 ml
Buffer E2	15 ml	170 ml	15 ml	170 ml
Buffer E3	15 ml	170 ml	15 ml	170 ml
Buffer E4	15 ml	170 ml	15 ml	170 ml
Buffer E5	15 ml	170 ml	15 ml	170 ml
Buffer PW2*	5 ml	50 ml	5 ml	50 ml
Elution Buffer (Endo-Free)	3 ml	30 ml	3 ml	30 ml
Buffer ER2	1.5 ml	6 ml	1.5 ml	6 ml
MaxPure EF Mini Column	4	50	4	50
2ml Collection Tubes	4	50	4	50
lysate Clear Midi Syringe	4	50	4	50
Extend Tubes	4	50	4	50
50ml Centrifuge Tubes	-	-	4	50
Support Tubes	-	-	4	50

Storage and Stability

The kit components can be stored dry at room temperature (15–25°C) and are stable for 24 months under these conditions. Buffer E2 and Buffer E4 may precipitate at low temperatures. Warm at 55°C until the precipitate is completely dissolved before use. After addition of RNase A, Buffer E1 is stable for 6 months when stored at 2–8°C.

Materials and Equipment to be Supplied by User

- Dilute Buffer PVW2 with 20ml (4 Preps) or 200ml (50 Preps) 100% ethanol and store at room temperature
- Add 0.2–0.5ml Buffer E1 to the RNase A powder, pipet to mix and then transfer to the Buffer E1 Buffer. The Buffer E1/RNase A mixture can be store at 2–8°C for 6 months.
- Heat Elution Buffer to 70°C if plasmid DNA is >10kb

Protocol

1. **Isolate a single colony from a freshly streaked selective plate and inoculate a culture of 25–50ml LB medium containing the appropriate selective antibiotic.** Incubate for 12–16 hours at 37°C with vigorous shaking. Centrifuge at 3000–5000 x g for 10 minute at room temperature. Decant or aspirate and discard the culture media.
It is strongly recommended that an endA negative strain of E. coli be used for routine plasmid isolation such as DH5a® and JM109®.
2. **Resuspend pelleted bacterial cells in 2.5ml Buffer E1/RNase A and transfer to a microcentrifuge tube.**
Ensure that RNase A has been added to Buffer E1. 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.
3. **Add 2.5ml Buffer E2 and mix thoroughly by inverting the tube 4–6 times.**
Mix gently by inverting the tube. Do not vortex, because this will result in shearing of genomic DNA and contamination of plasmid. If continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.
4. **Add 2.5ml Buffer E3. Mix immediately and thoroughly by inverting the tube 4–6 times.**
To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer E3. Large culture volumes (e.g., ≥ 5 ml) may require inverting up to 10 times. The solution should become cloudy.
5. Centrifuge at 3,000 ~5,000 x g for 5min.

6. Prepare a lysate Clear Midi Syringe by removing the plunger. Place the barrel in a tube rack to keep upright. Make sure the end cap is attached to the syringe tip. Immediately transfer the supernatant from Step 5 into the barrel of the Lysate Clear Midi Syringe.
7. Gently insert the plunger into the barrel to expel the cleared lysate into the 1.5 ml centrifuge tube. Add 1/3 volume of Buffer E4 to the clear Lysate. Invert 4~6 to mix.

Centrifuge Protocol (P1231B)

8. Combine MaxPure EF Mini Column with Sealing Ring, Support Tubes and Extender Tubes, then place it into 50ml collection tube. **Apply the mixture from step 7 to the Column.**
Centrifuge at 3,000 x g for 3min. Discard the flow through and reuse 50ml collection tube.
9. **Wash the column by adding 1ml Buffer E5** and centrifuging at 3,000 x g for 3 min.
10. **Wash the column by adding 2 ml Buffer PW2** and centrifuging at 3,000 x g for 3 min. Discard the flow through. Then follow Step 14 in below Vacuum Protocol.

Vacuum Protocol (P1231)

11. Combine Extender Tube with MaxiPure EF Column, then insert to Vacuum Manifold. **Apply the mixture from step 7 to the Column and applying the vacuum.**
12. Switch off the vacuum. Wash the Column by adding 1 ml of Buffer E5 to Column and applying the vacuum.
13. Switch off the vacuum. Wash the Column by adding 2 ml of Buffer PW2 to Column and applying the vacuum.
14. Transfer the MaxiPure EF Column into a 2.0ml Collection Tube(provided). Centrifuge at 10,000 x g for 2 min.
15. Place the Column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 100~300µl Elution Buffer (Endo-Free) to the center of each column, let stand for 1 min, and centrifuge for 1min.

Note: The plasmid DNA obtained in this step can be directly used for cell transfection.

By following the Additional Protocol, the endotoxin level can be reduced to ≤ 0.1 EU/ μ g, making the DNA suitable for animal injection applications. It can be used for animal injection or transfection of sensitive cells.

Additional Protocol:

Endotoxin-Free Plasmid DNA Purification (0.1 EU/ μ g DNA)

1. Transfer the plasmid DNA to a new 1.5 ml microcentrifuge tube. Add appropriate amount of Elution Buffer (Endo-Free) to reach a total volume of 500 μ l. Then add 60 μ l Buffer E1/RNase A, 60 μ l Buffer E3 and 60 μ l Buffer ER2, invert the tube 6 – 8 times.
2. Incubate on ice, or in a 2 – 8 °C refrigerator, for 10 – 15 minutes and invert several times.
At low temperatures, Buffer ER2 remains soluble and binds endotoxin molecules. Above 15° C, the Buffer ER2 – endotoxin complexes form insoluble micellar aggregates. If the room temperature is below 15° C, incubate the sample at 45 – 50 °C for 5 minutes after the ice incubation, until the solution becomes cloudy, and then proceed to the centrifugation in Step 3.
3. Centrifuge at 13,000 $\times$ g for 15 minutes at room temperature. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.
4. Add 0.7 volume isopropanol, invert 10 – 15 times, incubate for 10 minutes, then centrifuge at 13,000 $\times$ g for 15 minutes.
5. Carefully discard the supernatant. Add 1.0 ml 75% ethanol, vortex for 5 seconds, and centrifuge at 13,000 $\times$ g for 5 minutes.
6. Carefully discard the supernatant. Briefly centrifuge the tube and remove all residual liquid. Air-dry for 5 – 10 minutes.
7. Add an appropriate volume of Elution Buffer (Endo-Free), vortex to mix, and incubate at room temperature for 5–10 minutes to dissolve the plasmid DNA completely.