HiPure Plasmid EF Maxi Kit B

Introduction

This kit is designed to purify 100–1,500 µg of low-endotoxin plasmid DNA from 100–200 mL of bacterial culture. Its proprietary buffer system supports a broad range of plasmid vectors, including conventional high-, medium-, and low-copy plasmids as well as large constructs such as BACs, cosmids, and P1-derived vectors. DNA yield depends on plasmid copy number and culture volume. The purified plasmid DNA contains less than 0.1 EU/µg endotoxin and can be used directly for cell transfection, animal injection, and other endotoxin-sensitive applications.

Kit Contents

Product Number	P115601B	P115602B	P115603B
Purification Times	2 Preps	10 Preps	50 Preps
RNase A	100 µl	10 mg	50 mg
Buffer P1	20 ml	90 ml	450 ml
Buffer P2	20 ml	90 ml	450 ml
Buffer NS3	20 ml	90 ml	450 ml
Buffer ER2	6 ml	30 ml	150 ml
Buffer GWP	12 ml	60 ml	270 ml
Buffer EWB	12 ml	60 ml	270 ml
Buffer PW2*	6 ml	20 ml	100 ml
Elution Buffer (Endo-Free)	10 ml	30 ml	125 ml
Buffer CL	3 ml	12 ml	60 ml
Clear Maxi Syringe	2	10	50
HiPure DNA Maxi Column C	2	10	50
50 ml Collection Tube C	2	10	50

Storage and Stability

The kit components can be stored dry at room temperature (15–25°C) and are stable for at least 24 months under these conditions. If any precipitates form in the buffers, warm at 37°C 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

- Add 24ml (2 Preps), 80ml (10 preps) or 400ml (50 preps) 100% ethanol to the bottle of Buffer PW2 and store at room temperature
- Add 0.5 ml Buffer P1 to the vial of RNase A and pipette to dissolve. Transfer the entire solution to the Buffer P1 bottle. If RNase A is supplied as a liquid, briefly centrifuge the vial and transfer the entire contents to Buffer P1. Store the prepared Buffer P1/RNase A mixture at 2–8°C for up to 6 months.

Protocol: Low-Endotoxin Plasmid DNA Purification

1. **Inoculate a single colony into 1 ml LB medium containing the appropriate antibiotic in a 5–10 ml culture tube. Incubate at 37°C with shaking for 6–8 hours to prepare a starter culture.**
   Culture guidance: Aseptically pick a single colony with a sterile toothpick and inoculate 1 ml LB medium containing the appropriate antibiotic. Incubate at 37°C with shaking at 200–300 rpm for 6–8 hours. Plasmid loss may occur during glycerol-stock storage. Streak the glycerol stock onto an agar plate to recover the culture, and then use a sterile toothpick to pick a single isolated colony for starter culture preparation.

2. **Add 100–200 ml LB medium containing the appropriate antibiotic to a 0.5–1 L culture flask. Inoculate with 0.1% starter culture and incubate at 37°C with shaking for 12–16 hours. Centrifuge at 8,000 rpm for 5 minutes to harvest the bacterial culture.**
   Use a culture flask with a capacity at least 4–5 times the culture volume. A well-grown LB culture should have an OD600 of approximately 2.0–3.0, and the total bacterial biomass [OD600 × culture volume (mL)] should not exceed 600. Because bacteria grow rapidly in 2× YT or TB medium, these media may not allow sufficient time for plasmid replication and should be avoided when possible. If YT or TB medium is used, adjust the culture volume according to the bacterial biomass. For example, when the final OD600 is 10, use approximately 50–75 mL culture. The maximum binding capacity of the C7 purification column is 1,500 µg. Adjust the culture volume according to plasmid copy number.

3. Discard the culture medium and gently tap the inverted bottle on absorbent paper to remove residual liquid. Add 8 mL prepared Buffer P1/RNase A and completely resuspend the bacterial pellet by vigorous vortexing or pipetting.
   Complete resuspension is critical for optimal yield. No cell clumps should remain.

4. Add 8 mL Buffer P2 to the resuspended cells. Invert the tube 10–15 times to mix, incubate at room temperature for 2–3 minutes, and invert an additional 3–5 times during incubation until lysis is complete.
   Mix thoroughly by inversion. Do not vortex. After complete lysis, the solution should appear homogeneous and clear. When 200 mL of bacterial culture is processed, the lysate may become extremely viscous, representing a high-cell-density alkaline lysis condition. Increase the number of inversions and rotations, and shake gently as needed to ensure complete cell lysis. Do not allow the total lysis time to exceed 5 minutes.

5. Add 8 ml Buffer NS3 to the lysate. Immediately invert 15–20 times, or until the sample is thoroughly mixed and a flocculent suspension forms. Centrifuge at 8,000 rpm for 5 minutes.
   Mix immediately after adding Buffer NS3. After thorough mixing, a uniform white flocculent precipitate should form. When 200 mL culture is processed, the high-density lysate may produce large, compact precipitate aggregates during neutralization. Use additional inversions and rotations, with gentle shaking as needed, to break the aggregates into smaller pieces and allow Buffer NS3 to penetrate the precipitate completely.

6. Remove the plunger from the Clear Maxi Syringe and pour the supernatant into the syringe barrel. Reinsert the plunger and slowly express the cleared lysate into a 50 mL centrifuge tube.

7. Measure the volume of the cleared lysate. Add 0.1 volume Buffer ER2 and invert 10 – 15 times. Then add isopropanol equal to one-third of the cleared lysate volume and invert 10 – 15 times to mix.

8. Place a HiPure DNA Maxi Column C in a collection tube. Add 1.0 mL Buffer CL to the column and centrifuge at 8,000 rpm for 1 minute.
   Vacuum method: Order the optional VC2-10 accessory to perform Steps 8–13 more rapidly using vacuum filtration. Add 1.0 mL Buffer CL to the column, incubate for 3 minutes, and apply vacuum. Then sequentially add 5 ml sterile water, the mixture prepared in Step 7, Buffer GWP, Buffer EWB, and Buffer PW2 to the column, applying vacuum after each addition. After completing the Buffer PW2 wash in Step 12, add 5 ml absolute ethanol to the column and apply vacuum until all the liquid has passed through. Continue applying vacuum for 15 minutes to dry the column membrane, and then proceed to Step 14 for elution.

9. Transfer approximately 11 ml of the mixture to the column and centrifuge at 8,000 rpm for 1 minute. Discard the filtrate and return the column to the collection tube. Repeat for a total of three loads, until the entire mixture has passed through the column.
   The maximum column loading volume is 13 ml. Load the mixture in three portions.

10. Discard the filtrate and return the column to the collection tube. Add 5 ml Buffer GWP to the column, incubate for 2 minutes, and centrifuge at 8,000 rpm for 1 minute.

11. Discard the filtrate and return the column to the collection tube. Add 5 ml Buffer EWB to the column and centrifuge at 8,000 rpm for 1 minute.

12. Discard the filtrate and return the column to the collection tube. Add 9 ml prepared Buffer PW2 to the column and centrifuge at 8,000 rpm for 10 minutes.

13. Remove the column without allowing the bottom of the column to contact the filtrate. Open the cap and dry the column membrane at room temperature for 10 – 15 minutes. Discard the liquid from the collection tube and tap the tube several times on absorbent paper to remove residual liquid. Allow the tube to dry before reuse.
   The 80% ethanol in prepared Buffer PW2 provides a soaking disinfection step. After the filtrate has been discarded and the tube has dried, Collection Tube C may be reused in Step 14 to collect the plasmid DNA.

14. Place the column in Collection Tube C. Add 1.0 mL Elution Buffer (Endo-Free) directly to the center of the column membrane. Incubate for 2 minutes and centrifuge at 8,000 rpm for 3 minutes.
   This elution recovers approximately 60–75% of the bound DNA. For the highest DNA concentration, omit the second elution in Step 15 or collect the two eluates separately.

15. Add 0.7 mL Elution Buffer (Endo-Free) directly to the center of the column membrane. Incubate for 2 minutes and centrifuge at 8,000 rpm for 3 minutes. Transfer the plasmid DNA to a new 2 mL microcentrifuge tube and store at −20° C.
   A precipitate may form during storage of plasmid DNA. Remove it by centrifugation if necessary.

Additional Protocol:

Endotoxin-Free Plasmid DNA Purification (0.1 EU/μg DNA)

1. Transfer the plasmid DNA to a 2.0 mL microcentrifuge tube. Add 0.1 volume Buffer NS3 and 0.1 volume Buffer ER2, and invert the tube several times.
Example: For 1.5 mL DNA solution, add 150 μL each of Buffer NS3 and Buffer ER2.

2. Incubate on ice, or in a 2 – 8° C refrigerator, for 10 – 15 minutes.
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 laboratory 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 × g for 15 minutes at room temperature. Transfer the supernatant to a new microcentrifuge tube.

4. Add 0.7 volume isopropanol, invert 10 – 15 times, incubate for 10 minutes, and centrifuge at 13,000 × g for 15 minutes.
For easier handling, divide the mixture among several 1.5 – 2.0 mL microcentrifuge tubes.

5. Carefully discard the supernatant. Add 1.0 mL 75% ethanol, vortex for 5 seconds, and centrifuge at 13,000 × g for 3 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.