E.Z.N.A.® FastFilter Plasmid DNA Maxi Kit

Revision No: 2.0

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	D6924-00	D6924-03	D6924-04
Purifications	2	25	100
HiBind® DNA Maxi Columns	2	25	100
50 mL Collection Tubes	2	25	100
Lysate Clearance Filter Syringe	2	25	100
Solution I	30 mL	270 mL	1050 mL
Solution II	30 mL	270 mL	1050 mL
N3 Buffer	15 mL	135 mL	530 mL
GBT Buffer	20 mL	230 mL	900 mL
HBC Buffer	18 mL	200 mL	3 x 250 mL
DNA Wash Buffer	15 mL	150 mL	3 x 200 mL
RNase A	120 µL	1.2 mL	5 mL
Elution Buffer	15 mL	150 mL	2 x 300 mL

Supplied by user:

- 100% ethanol
- 100% isopropanol
- Refrigerated centrifuge with swing bucket rotor capable of 4,000 x *g*
- Vortexer
- Appropriate centrifuge bottle for Step 1
- Nuclease-free 50 mL centrifuge tubes
- Ice bucket
- Optional: Water bath, incubator, or heat block capable of 70°C
- Optional: 3M NaOH for Column Equilibration
- Optional: 3M NaOAc (pH 5.2) and 70% ethanol for DNA Precipitation protocol

Before starting:

- Prepare Solution I, HBC Buffer, and DNA Wash Buffer according to the directions on the bottles.
- Chill N3 Buffer on ice.
- Check Solution II and GBT Buffer for precipitation before use. Redissolve any precipitation by warming to 37˚C.
- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb.

Plasmid DNA Extraction and Purification from 50-200 mL E. coli Culture

LYSE

1. Transfer 50-200 mL overnight culture to an appropriate centrifuge bottle (not provided).
2. Centrifuge at 4,000 x *g* for 10 minutes at room temperature. Decant or aspirate and discard the culture media. Use a clean paper towel to blot excess liquid from the wall of the bottle.
3. Add 10 mL Solution I mixed with RNase A. Vortex or pipet up and down to completely resuspend the cells.
4. Add 10 mL Solution II. Invert and rotate the tube gently 10 times to obtain a cleared lysate. This may require a 2 minute incubation at room temperature with occasional mixing. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes.
5. Add 5 mL cold N3 Buffer. Invert and rotate the tube gently until flocculent white precipitates form. This may require a 2 minute incubation at room temperature with occasional mixing.

   Note: The solution must be mixed thoroughly. This is vital for obtaining good yields. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.
6. Add 8.3 mL GBT Buffer. Gently invert the tube 4 times.
7. Prepare a Lysate Clearance Filter 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.
8. Immediately transfer the lysate from Step 6 into the barrel of the Lysate Clearance Filter Syringe. Let sit at room temperature for 2 minutes. Any white precipitates should float to the top.
9. Hold the Lysate Clearance Filter Syringe barrel over a 50 mL centrifuge tube (not provided) and remove the end cap from the syringe tip. Gently insert the plunger into the barrel to expel the cleared lysate into the 50 mL centrifuge tube. Some of the lysate may remain in the flocculent precipitate. DO NOT force this residual lysate through the filter.

BIND

Note: Steps 10-20 should be performed in a swing bucket rotor for maximum plasmid DNA yield. All of centrifugation steps should be carried out at room temperature.

10. Insert a HiBind® DNA Maxi Column into a 50 mL Collection Tube (provided).

OPTIONAL: Protocol for Column Equilibration

1. Add 3 mL 3M NaOH to the HiBind® DNA Maxi Column.
2. Let sit at room temperature for 4 minutes.
3. Centrifuge at 4,000 x *g* for 3 minutes.
4. Discard the filtrate and reuse the collection tube.

11. Transfer 15 mL cleared supernatant from Step 9 by aspirating it into the HiBind® DNA Maxi Column. Be careful that no cellular debris is transferred to the HiBind® DNA Maxi Column.

12. Centrifuge at 4,000 x *g* for 3 minutes. Discard the filtrate and reuse the collection tube.

13. Repeat Steps 11-12 until all of the cleared supernatant has been transferred to the HiBind® DNA Maxi Column.

WASH

14. Add 10 mL HBC Buffer diluted with 100% isopropanol (see the bottle for instructions). Centrifuge at 4,000 x *g* for 3 minutes. Discard the filtrate and reuse the collection tube.

15. Add 15 mL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at 4,000 x *g* for 3 minutes. Discard the filtrate and reuse the collection tube.

16. Add 10 mL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at 4,000 x *g* for 3 minutes. Discard the filtrate and reuse the collection tube.

17. Centrifuge the empty HiBind® DNA Maxi Column at 4,000 x *g* for 10 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

ELUTE

18. Transfer the HiBind® DNA Maxi Column to a nuclease-free 50 mL centrifuge tube (not provided).

19. Add 1.5-3 mL Elution Buffer directly to the center of the column matrix. Let sit at room temperature for 3 minutes.

20. Centrifuge at 4,000 x *g* for 5 minutes. Store DNA at -20°C.

DNA Precipitation

The concentration of the eluted plasmid DNA varies with copy number, host strain, and growth conditions. In some cases, residual ethanol may also be present. To adjust the DNA concentration following plasmid DNA elution or for the removal of residual ethanol, perform the following isopropanol precipitation protocol.

1. Carefully transfer the eluted plasmid DNA to a clean tube suitable for precipitation. Add 1/10 volume 3M NaOAc (pH 5.2) and 0.7 volumes 100% isopropanol (room temperature). Vortex to mix.

2. Centrifuge at ≥15,000 x *g* for 20 minutes at 4°C. Carefully decant the supernatant.

3. Add 1-2 mL 70% ethanol. Vortex to resuspend the pellet.

4. Centrifuge at ≥15,000 x *g* for 10 minutes at 4°C. Carefully decant the supernatant.

5. Air dry the pellet for 10 minutes.

6. Add 200-500 µL Elution Buffer. Store DNA at -20°C.