E.Z.N.A.® Plasmid DNA Maxi Kit

Revision No: 1.1

Quick Guide

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

Product	D6922-00	D6922-01	D6922-02	D6922-04
Purifications	2	5	20	100
HiBind® DNA Maxi Columns	2	5	20	100
50 mL Collection Tubes	2	5	20	100
Solution I	30 mL	70 mL	270 mL	2 x 675 mL
Solution II	30 mL	70 mL	270 mL	2 x 675 mL
Solution III	35 mL	100 mL	2 x 170 mL	2 x 850 mL
HBC Buffer	25 mL	40 mL	2 x 80 mL	3 x 250 mL
DNA Wash Buffer	25 mL	50 mL	3 x 50 mL	4 x 200 mL
RNase A	100 µL	300 µL	1.2 mL	2 x 3 mL
Elution Buffer	15 mL	60 mL	250 mL	2 x 500 mL

Supplied by user:

- 100% ethanol
- 100% isopropanol
- Centrifuge with swing bucket rotor capable of 4,000 x *g*
- Centrifuge capable of 15,000 x *g*
- Nuclease-free 50 mL centrifuge tubes
- 30 mL or 50 mL centrifuge tubes capable of withstanding 15,000 x *g*
- Appropriate centrifuge bottle for Step 1
- Optional: Water bath, incubator, or heat block capable of 65°C
- Optional: Sterile deionized water
- Optional: 3M NaOH for Column Equilibration
- Optional: 3M NaOAc for DNA Precipitation protocol

Before starting:

- Prepare Solution I, DNA Wash Buffer, and HBC Buffer according to the directions on the bottles.
- Check Solution II and Solution III for precipitation before use. Redissolve any precipitation by warming to 37˚C.
- Heat Elution Buffer to 65°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 12 mL Solution I mixed with RNase A. Vortex or pipet up and down to completely resuspend the cells.
4. Transfer the cell suspension to a 30 mL or 50 mL centrifuge tube capable of withstanding 15,000 x *g* (not provided).
5. Add 12 mL Solution II. Invert and rotate the tube gently 10-12 times to obtain a cleared lysate. This may require a 2-3 minute incubation at room temperature with occasional mixing.

   Note: 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. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.
6. Add 16 mL Solution III. Invert and rotate the tube gently until flocculent white precipitates form. This may require a 2-3 minute incubation at room temperature with occasional mixing.

   Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.
7. Centrifuge at 15,000 x *g* for 10 minutes (preferably at 4°C). A compact white pellet will form. Promptly proceed to the next step.

BIND

8. Insert a HiBind® DNA Maxi Column into a 50 mL Collection Tube (supplied).

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.

BIND

9. Transfer 20 mL cleared supernatant from Step 7 by CAREFULLY aspirating it into the HiBind® DNA Maxi Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Maxi Column.
10. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.
11. Repeat Steps 9-10 until all of the cleared supernatant has been transferred to the HiBind® DNA Maxi Column.

WASH

12. Add 10 mL HBC Buffer diluted with 100% isopropanol (see the bottle for instructions). Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.
13. Add 15 mL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.
14. Repeat Step 13 for a second DNA Wash Buffer wash step.
15. 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

16. Transfer the HiBind® DNA Maxi Column to a nuclease-free 50 mL centrifuge tube (not supplied).
17. Add 1.5-3 mL Elution Buffer or sterile deionized water directly to the center of the column matrix.
18. Let sit at room temperature for 5 minutes.
19. Centrifuge at 4,000 x *g* for 5 minutes.
20. 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 4ºC. 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 4ºC. Carefully decant the supernatant.
5. Air dry the pellet for 10 minutes.
6. Add 200-500 µL Elution Buffer. Store DNA at -20°C.