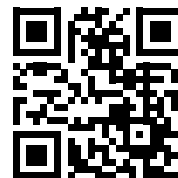


## Quick Guide

Please visit [www.omegabiotek.com](http://www.omegabiotek.com) for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.



Product	D6904-00	D6904-03	D6904-04
Purifications	2	25	100
HiBind® DNA Midi Columns	2	25	100
15 mL Collection Tubes	2	25	100
Solution I	8 mL	70 mL	270 mL
Solution II	8 mL	70 mL	270 mL
Solution III	8 mL	100 mL	400 mL
HBC Buffer	5 mL	58 mL	250 mL
DNA Wash Buffer	5 mL	40 mL	200 mL
RNase A	25 µL	300 µL	1.2 mL
Elution Buffer	5 mL	60 mL	220 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 15 mL and 50 mL centrifuge tubes
- 30 mL or 50 mL centrifuge tubes capable of withstanding 15,000 x g
- Optional: Water bath, incubator, or heat block capable of 65°C
- Optional: Sterile deionized water
- Optional: 3M NaOH for Column Equilibration
- Optional: 3M NaOAc (pH 5.2) 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 20–50 mL *E. coli* Culture

1. Transfer 20-50 mL overnight culture to a 50 mL centrifuge tube (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 tube.
3. Add 2.25 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 2.25 mL Solution II. Invert and rotate the tube gently 8-10 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<sub>2</sub> in the air.

6. Add 3.2 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.

LYSE

BIND

8. Insert a HiBind® DNA Midi Column into a 15 mL Collection Tube (provided).

### OPTIONAL: Optional Protocol for Column Equilibration

1. Add 1 mL 3M NaOH to the HiBind® DNA Midi 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.

WASH

9. CAREFULLY transfer 3.5 mL cleared supernatant from Step 7 to the HiBind® DNA Midi Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Midi Column.
10. Centrifuge at 4,000 x *g* for 3 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 Midi Column.
12. Add 3 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.
13. Add 3.5 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.
14. Repeat Step 13 for a second DNA Wash Buffer wash step.

ELUTE

15. Centrifuge the empty HiBind® DNA Midi 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.
16. Transfer the HiBind® DNA Midi Column to a nuclease-free 15 mL centrifuge tube (not provided).
17. Add 0.5-1 mL Elution Buffer or sterile deionized water directly to the center of the column matrix.
18. Let it sit at room temperature for 3 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 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.