E.Z.N.A.® Plasmid DNA Mini Kit II

Revision No: 3.0

Quick Guide

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

Product	D6945-00	D6945-01	D6945-02
Purifications	5	50	200
HiBind® DNA Mini Columns II	5	50	200
2 mL Collection Tubes	5	50	200
Solution I	5 mL	30 mL	120 mL
Solution II	5 mL	30 mL	120 mL
Solution III	5 mL	40 mL	2 x 80 mL
HBC Buffer	5 mL	25 mL	80 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL
RNase A	Pre-Added	100 µL	400 µL
Elution Buffer	2 mL	30 mL	30 mL

Supplied by user:

- Microcentrifuge capable of 13,000 x g
- Centrifuge with swing buckets capable of 5,000 x g
- Vortexer
- Nuclease-free 1.5 mL and 2 mL microcentrifuge tubes
- Appropriate centrifuge tubes for Step 2
- 100% ethanol
- 100% isopropanol
- Optional: sterile deionized water
- Optional: water bath, heat block, or incubator capable of 70°C
- Optional: 3M NaOH solution

Before starting:

- Prepare Solution I, HBC Buffer, and DNA Wash Buffer according to the directions on the bottles.
- Set water baths, heat blocks, or incubators to 55°C and 70°C.
- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb.
- Check Solution II and Solution III for precipitation before use. Redissolve any precipitation by warming to 37˚C.

Plasmid DNA Extraction and Purification from 10-15 mL E. coli culture

LYSE

1. Grow 10-15 mL culture overnight in an appropriately size culture tube or flask.
2. Centrifuge at 5,000 x g for 10 minutes at room temperature. Decant or aspirate and discard the culture media.
3. Add 500 µL Solution I mixed with RNase A (see the bottle for instructions). Vortex to completely resuspend pellet and mix thoroughly. Transfer suspension into a new 2 mL microcentrifuge tube.
4. Add 500 µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary. Avoid vigorous mixing and do not exceed a 5 minute incubation.
5. Add 700 µL Solution III. Immediately invert several times until a flocculent white precipitate forms. Centrifuge at maximum speed (≥13,000 x g) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.
6. Insert a HiBind® DNA Mini Column II into a 2 mL Collection Tube.

BIND

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column II.
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

7. Transfer 700 µL cleared supernatant from Step 5 by CAREFULLY aspirating it into the HiBind® DNA Mini Column II. Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.
8. Repeat Step 7 until all cleared lysate has been transferred to the HiBind® DNA Mini Column II.
9. Add 500 µL HBC Buffer diluted with 100% isopropanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.

WASH

10. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.

Optional: Repeat Step10 for a second DNA Wash Buffer wash step.

11. Centrifuge the empty HiBind® DNA Mini Column II at maximum speed for 2 minutes to dry the HiBind® DNA Mini Column II. This step is critical for removal of trace ethanol that may interfere with downstream applications.

ELUTE

12. Transfer the HiBind® DNA Mini Column II into a nuclease-free 1.5 mL microcentrifuge tube.

13. Add 80-100 µL Elution Buffer or sterile deionized water. Let sit at room temperature for 60 seconds. Centrifuge at maximum speed for 1 minute.

14. Store eluted DNA at -20°C.