Quick Guide

VWR LIFE SCIENCE

peqGOLD Plasmid Miniprep Kit II

Product	13-6945-00	13-6945-01	13-6945-02
Purifications	5	50	200
peqGOLD 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*
- Vortexer
- Centrifuge with swing buckets capable of 5,000 x *g*
- Appropriate culture tubes/flasks
- 1.5 and 2 mL microcentrifuge tubes
- 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 bath, heat block, or incubator to 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 Purification from 10-15 mL *E. coli* culture

1. Grow 10-15 mL culture overnight in an appropriately sized 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 (not provided).
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 peqGOLD DNA Mini Column II into a 2 mL Collection Tube.

   Optional Protocol for Column Equilibration:

   1. Add 100 µL 3M NaOH to the peqGOLD 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 peqGOLD 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 peqGOLD 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.

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

12. Transfer the peqGOLD DNA Mini Column II into a nuclease-free 1.5 mL microcentrifuge tube (not provided).

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.

    Note: Heat Elution Buffer to 70°C if plasmid DNA is >10 kb.

14. Store eluted DNA at -20°C.