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# 4-200™ plasmid purification kit.

**Protocol**

**Prior to using the kit for the first time, add 8 mL of 96% ethanol to the bottle labeled “Solution #3”.**

**Store the kit in the refrigerator.**

1. Collect cells from 1.5 mL of overnight *E. coli* cell culture by centrifugation in micro-centrifuge (13 rsf) for 2 minutes and remove supernatant (by opening and inverting the tube, then giving it 1 – 2 intense shakes down into a waste receptacle).
2. Re-suspend cells in the remaining (~30 µl) supernatant using a Vortex.
3. Add 135 µl of **Solution #1** per tube.
4. Thoroughly mix the contents of tubes (shaking tubes 2 – 3 times should suffice), and place the tubes at 37oC for 5 – 15 minutes.
5. Transfer tubes to 80oC and continue incubation for an additional 15 minutes.
6. Centrifuge tubes for 10 – 25 minutes at 13 rsf.
7. Remove pellets from tubes by inserting a sterile toothpick (provided in the kit) into each pellet, spinning it inside of pellet, and pulling the toothpick out of the tube together with the stuck pellet. *Note*: We recommend using metal forceps sterilized by flame for taking toothpicks out of the storage container.
8. Add 145 µl of **Solution #2** per tube to the remaining supernatant and mix the contents by gently inverting the tubes several times (for plasmids larger than 20 k.b.) or by intensely shaking 2 – 3 times.
9. Centrifuge tubes for 10 minutes at 13 rsf.
10. Remove supernatant by aspiration.  
    *Note*: We recommend using faucet aspirator/vacuum pump).
11. Add 50 µl of **Solution #3** (remember to add ethanol) per tube.
12. Centrifuge tubes for 2 minutes at 13 rsf.
13. Remove supernatant by aspiration.
14. Add 50 µl of **Solution #4**.
15. Incubate at 37oC for 5 – 10 minutes.
16. The purified DNA is ready for further manipulations!