

Quick Protocol Reference Card Spin Doctor Plasmid & BAC Prep Kits

Midi: 50 mL Culture

Midi-Maxi: 125 mL Culture

Maxi: 250 mL Culture

Mega: 500 mL Culture

Protocol

1. Spin down pellet, _____RPM (4000g) 10 minutes 4° C.
2. Re-suspend in Solution 1: **5mL/10mL/20mL/40mL**.
3. Add **5mL/10mL/20mL/40mL** Solution 2, invert 20 times.
4. Add **5mL/10mL/20mL/40mL** Solution 3, invert 20 times.
5. Centrifuge at _____RPM (8k-13k g) (4.5k-13k g in Falcon Tubes) 10 minutes 4° C.
6. Strain supernatant through a Falcon Filter, into a clean centrifuge bottle.
7. Add **15mL/30mL/60mL/120mL** Isopropanol and gently invert 20 times.
8. Centrifuge at _____RPM (8k-13k g) (4.5k-13k g in Falcon Tubes) 10 min 4° C.
9. Discard supernatant, air dry 10 min.
You may store pellet at -20° C indefinitely
10. Re-suspend Pellet in **500uL/500uL/1mL/2mL** of Solution 4 and transfer into **1/1/2/4** RNase Clear Tube(s).
11. Incubate 20 minutes in a 37° C Water bath, inverting half way through.
You may store pellet at -20° C indefinitely
12. Add **800 uL** of Solution 5 to each tube, invert 30 times.
13. Add **600 uL** of Isopropanol to each tube, invert 20 times. Microfuge at maximum speed for 5 minutes. Discard supernatant.
14. Wash DNA pellet once with 1 mL of 70% Ethanol, discard supernatant and air dry 5 minutes.
15. Re-suspend pellet using 800 uL of Buffer N. Incubate 5 minutes at 57°C, then pipette 5-10 times. Repeat incubation if necessary.
16. Add 900 uL of Isopropanol to each tube, mix by gently inverting 20 times. Centrifuge at maximum speed for 5 minutes. Decant.
17. Re-suspend each prep tube in 500 uL of Buffer N by pipetting 8-10 times and incubating for 5 minutes at 57°C. Add 800 uL of Solution 7 to each tube and mix by inverting 30 times. Add 600 uL of Isopropanol to each tube, invert 20 times, and centrifuge at maximum speed for 5 minutes. Decant supernatant.
18. Wash pellet twice with 1 mL of 70% Ethanol.
21. Pipette off residual Ethanol, air dry for 10 minutes
22. Re-suspend DNA pellet in 10 mM Tris buffer, pH 8.0.
 - a. Add **250uL/500uL/375uL/250uL per tube**.
 - b. Incubate 57° C for 10-20 minutes, pause every 5 minutes during incubation to re-suspend with pipet.
 - c. After re-suspension you can re-combine the tubes from maxi and mega preps into a single tube.
23. Optional: Microfuge at full speed 5 minutes, draw 900 uL off the top and transfer to a clean tube.

Additional Steps for Spin Doctor Super Clean Kit

1. At room temperature, pre spin the mini-column in a microfuge at _____RPM (1000g) for 3 minutes.
2. Transfer the mini-column a 1.5 ml collecting tube supplied with the kit, carefully load the sample to the center of the gel bed surface.
3. Centrifuge at _____RPM (1000g) for 3 minutes.
4. Discard the mini-column and retain the purified sample from the bottom of the collecting tube.