Spin columns preparation and use

Prepare column.
1. Prepare Sephadex. Hydrate Sephadex. Incubate 50 g Sephadex G-25 sit in 400 mL buffer* at room temperature for 3 h or heated in water bath at 90 °C for 1 h.
2. Roll up glass wool and stuff into 1 mL plastic syringe. The amount of glass wool should fit into 0.1 mL.
3. Place the syringe in a 13 x 100 mm test tube.
4. Pipet the slurry of Sephadex into the column and let drain.
5. After the gel settles, add more.
6. Centrifuge for two minutes.
7. Add more Sephadex and centrifuge for 10 min.

De-salting
8. The buffer can be exchanged by eluting two mL through column.
9. Centrifuge for 10 min.
11. Place the syringe on top so that the eluant will flow into the centrifuge tube.
12. Add 100 μL of sample. If sample is less than 100 μL, first load the sample and then add additional buffer to add a total of 100 μL to the column.

Test spin column and centrifuge speed
1. Prepare two solutions (1) 50 mg/mL Blue Dextran and (2) 50 mg/mL bromophenol blue in water.
2. Load 100 μL each solution on top of spin column.
3. Centrifuge 10 min.
4. The Blue Dextran should all come through.
5. Bromophenol blue should stay in the column.

* This buffer will be the buffer that the desired molecule will be dissolved in after the procedure.

Typically this would be 50 mM Tris-HCl (pH 8.0), 1 mM EDTA