Protocol for PC12 Transfection

Kit: Invitrogen Lipofectamine 2000 Reagent
Reference: Standard Protocol for lipofectamine 2000 from Invitrogen

Chemicals:
- Lipofectamine 2000
- Opti-MEM /w Glutamax
- PC12 Medium (DMEM /w P/S + 10% HIHS + 5% FCS)

Procedures:
1. Plate PC12 cells in 35mm poly-D-lysine coated dish at 1:16 dilution from 70-80% confluent 90mm culture 24 hours before transfection
2. When culture reaches 60-70% confluent, they are good for transfection
3. Optional: Pretreat PC12-cells in OPTI-MEM for O/N prior to transfection (reduce toxicity)
4. Add 2ug plasmid to 250ul OPTI-MEM in a tube
5. Add 10ul lipofectamine 2000 to 250ul OPTI-MEM in another tube
6. Incubate both for 5 minutes at room temperature
7. Mix and incubate for a further 30 minutes
8. Aspirate medium in culture dish and add transfection mixture
9. Incubate in 37C incubator for 4-6 hours
10. Replace OPTI-MEM with PC12 medium and let growth for 24-48 hours.