

### **Lipofectamine2000 for PC12 transfection**

1. The day before transfection, count the cells, plate them in 6-well plates at  $2 \times 10^5$  cells per well. Cells are plated in 1.5 ml of their normal growth medium containing serum and without antibiotics.
2. For each well of cells to be transfected, dilute 0.9  $\mu\text{g}$  of the desired DNA plus 0.1  $\mu\text{g}$  GFP into 1 ml DMEM(HG), No serum, No P/S and mix well.
3. For each well of cells, add 3  $\mu\text{l}$  of the lipofectamine-2000 reagent into the DNA containing medium, vortex (at speed 5-6) within 5 min, then incubate for at least 20 min to allow the DNA-LF2000 reagent complexes to form.
4. During the 20 min, wash cells once with DMEM(HG), No serum, No P/S.
5. Add the DNA-LF2000 reagent complexes (1 ml) directly to each well and mix gently by rocking the plate back and forth.
6. Incubate the cells at  $37^\circ\text{C}$  in a  $\text{CO}_2$  incubator for 1h, add 1 ml of DMEM containing 20% horse serum/10% FBS into each well. And then incubate the cells at  $37^\circ\text{C}$  in a  $\text{CO}_2$  incubator for a total of 24h.
7. Remove the DNA-LF2000 medium, PBS wash once and add fresh culture medium containing the desired reagent (ex with 100 ng/ml NGF).
8. Incubate the cells for 3 more days and assess neurite outgrowth under a microscope.

Growth medium: DMEM(HG) + 10% HS + 5% FBS + P/S

For 24 well, use 1/5 vol of all the reagents, plating cells with more volume of growth medium (0.3-0.4 ml/well).