Transfection

Description

Materials

Optimum in fridge, DNA in freezer, lipofectamine in fridge.

Total time

6.5 hours

Procedures

Lipofectamine 2000 method (Invitrogen) (for 1 well in 6-well cluster 10cm2, small dish of 35 mm).

1. The day before transfection, pass confluent cell 1:3 in 10%FBS without Penicillin/Straptomyosin.
2. Check the cell condition, if it is in 60-80% confluency, proceed, otherwise, start a new experiment.
3. 1 ug DNA gently mixed into 100 ul Optimum only, gently tap the tip of vial to mix.
4. 2 ul lipofectamine mixed into 100 ul Optimum only, gently tap the tip of vial to mix. (1ug DNA – 2ul lipofectamine)
5. Wait for 5 min, gently apply lipofectamine-Optimum to DNA-Optimum. Tilt and rotate the DNA tube, while adding lipo-optimum drop by drop on its wall.
6. Tap or reverse the tube gently to mix.
7. Incubate in RT for 20 min for the complex formation between DNA and lipofectamine
8. Apply the DNA-Lipofectamine complex Optimum to cells. Gently swirl the dish to mix.
9. Incubate 5.5 hr in incubator. check cell toxicity, if severe, stop the incubation and apply fresh 0.5% FBS-DMEM, if not, continue incubation until next day and change to 0.5%FBS DMEM.

Notes

The optimal time interval between passing cells and transfection is 15-18 hours; and that between transfection and imaging is 36-54 hours.

When getting vials, don’t touch it with hand. Put only pipette tip in containers, not any part of pipette.