

96-well Plate Dual Luciferase Assay

Reagents:

1. Plasmids: can be found in my plasmid(1) box in the -20°C freezer.
985: FR-tk-luciferase
2517: Renilla-tk-luciferase
2145: GFP
2. Lipfectamine and lipofectamine **plus** reagents: in the upper shelf of the medium freezer in tissue culture room.
3. DMEM-10
4. D20F: 100ul for one well

Protocol:

1. I pick up colonies and grow them in 24well plate. One ml medium each well.
2. Once the cells of most of the wells are more than 30% confluent, I will take out 700 ul of the medium and resuspend the cells using the 1ml pipette.
3. Transfer about 50 ul to 200 ul out of 300ul to the 96 well plate depending on the confluency of each well. (just to make sure the cell numbers of different wells are within 4 fold range)
4. Spin down the cells in 96 well plate at 1200 rpm for 10 min.
5. Preparing the DNA mixture for lipofectamine transfection:
 - a. for one transfection, I use 100 ng of 985 and 100ng of 2517 and 25ng of 2145.
 - b. Prepare LipoA: for one transfection, I use 50 ul of serum free medium(e.g. DMEM-10) and 1.5 ul of lipofectaming **plus** reagent and DNA. Please mix the DNA and DMEM-10 before adding Lipo plus reagent.
 - c. Prepare Lipo B: for one transfection, I use 50 ul of DMEM-10 and 1.5 ul of lipofectamine reagent.
 - d. Mix A and B.
 - e. Let sit at Rt for >15'
6. Suck off the medium of the 96 wells and add 100 ul of mixture (step 5e) by multichanel pipette.
7. Let sit in incubator for >1.5 hours.
8. Add 100 ul of D20F and let it grow for two days.

Sugden Lab.

Dual luciferase assay:

Reagents:

Dual luciferase kit is in the bottom shelf of the freezer containing Mark and Greg's stuff. The manual can be found in my bench drawer.

1. Suck off the medium from 96 well plates and wash with PBS once. Add 50 ul of 1X passive lysis buffer to each well and pipette up and down.
2. Prepare LARII by solving the luciferase substrate powder with the corresponding buffer (10ml for one bottle of powder).
Prepare Stop&Glo.
3. take 10 ul to measure luciferase activity using program #5. Injector #1 is for LARII and injector #2 is for Stop& Glo.