Dual Luciferase Assay

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ABSTRACT

Dual luciferase assay WIP

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MATERIALS TEXT

1. Dual-Luciferase® Reporter Assay System found here.
2. FuGENE transfection reagent found here.

1 Cell culture

- If you are reading this, I am assuming that you already have 100,000 cells plated in each well of a 24-well TC plate (or something of equal measure)
- Let them rest overnight because tomorrow you will transfect them

2 Transfection (Transient) of Plasmid

- Dual luciferase assays work by expressing and measuring two luciferase plasmids: Firefly Luciferase and Renilla luciferase, which helps normalize transfection efficiencies of the plasmids
- Assemble the following mixture for a 24 well plate:

2uL of FuGENE
1uL plasmid A (200ng)
1uL plasmid B (200ng)
50uL Optimem

- Let sit for max 15 minutes
- Add dropwise to the cells
- Leave for 24 hours

3 Cell Lysing and Luciferase Assay

- Remove media from each well and wash with PBS; remove the PBS
- Add 50uL trypsin to each well and coat every cell
- Leave in incubator for 3 minutes
- Pipette all cells off of plate and add cells to 1.5mL tubes
- Spin down cells at 1,500RPM 4C for 5 minutes to pellet cells
- Remove supernatant
- Re suspend cells in 50uL of PLB 1X Passive Lysis Buffer (PLB)
- Incubate lysates on ice for at least five minutes
- Vortex each tube for 10-15 seconds
- Incubate for 5-10 more minutes on ice
- Centrifuge the tubes at 12,000 rcf at 4C for two minutes
- Transfer the supernatant of each tube to a new labeled tube.
- Either store the lysates at -80C or continue to the luciferase assay

4 The Luciferase Assay Itself

- Set up a GloMax luminometer to measure for 10 seconds
- For each sample you want to measure, add 20 uL of LARII and 20 uL lysate to a microfuge tube
- Vortex the tube briefly
- Put the tube in the slot in the luminometer, close the lid, and measure
- Usually measure each sample two times