LUCIFERASE ASSAY: (PROMEGA SYSTEM)
(24 Well Plate)

Cell Lysis

1. Thaw 5X PLB and prepare appropriate amount of 1X PLB (100 µl per well) by diluting in dH20
2. Remove media from cells by aspiration
3. GENTLY wash with 1X PBS (perhaps should be skipped for 293T cells)
4. Add 100 µl 1X PLB to each well. Place on shaker at RT for 30 min.

Reagent Preparation

5. Prepare Luciferase assay reagent II

   Resuspend lyophilized Luciferase Assay Substrate in 10 mL of Luciferase Assay Buffer II and label "LARII"
   (stable for one year at –80)
   * need 100 µl per assay, plus about 2 mLs to tube volume for luminometer

Luminescence measurement

6. Read samples in Molecular Devices "LMax" luminometer

   Dispense 20 µL cell lysate into white opaque luminometer microtiter plate
   Prime P injector (7 injections) with LARII
   Inject 100 µl LARII per well with P-injector.
   Read with a 2 sec premeasurement delay, 10 sec integration to detect firefly luciferase.

Wash injector

Place P injector tube into a 50ml Falcon tube containing ddH_2O. Select **Wash Injectors…** from the **Control** menu. Accept the default settings (30 injections) and click **OK**.

Repeat this wash step with 70%EtOH (30 injections). Perform a final "Wash" that will just dry the injector tube.

Protein quantitation

Quantitate protein concentrations for each well by Bio-Rad assay.

Data analysis

Normalize luciferase numbers to protein concentration numbers.

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