DUAL LUCIFERASE REPORTER SYSTEM (PROMEGA)
(24 well plate)

Transfections

Cells should be transfected with a test reporter (firefly luciferase) and a normalization reporter driven by a constitutive promoter (renilla luciferase)
PRL-TK (thymidine kinase promoter) works well at a ratio of 1:200.

Cell Lysis

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

Reagent Preparation

1. 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 mL to tube volume for luminometer

2. Prepare Stop and Glo:

   a) Make 50X Stop and Glo substrate: Transfer 200 µl Stop and Glo substrate solvent into lyophilized Stop and Glo substrate, vortex (stable for one year at –80).

   b) Make 1X Stop and Glo substrate: Add 1 vol of 50X Stop and Glo substrate to 50 vol Stop and Glo buffer. Store in dark glass bottle.

   * need 100 µl per assay, plus about 2 mLs to tube volume for luminometer

Luminescence measurement

Read samples in Molecular Devices "LMax" luminometer:

1. Dispense 20 µL cell lysate into white opaque luminometer microtiter plate.
2. Prime P and M injectors (7 injections each) with LARII and Stop and Glo.
3. Inject 100 µl LARII per well with P-injector. Read with a 2 sec premeasurement delay, 10 sec integration to detect firefly luciferase.
4. Inject 100 µl Stop and Glo per well with the M injector. Read with a 2 sec premeasurement delay, 10 sec integration to detect renilla luciferase. Print out data.

Wash injectors

1. Place <u>both</u> tubes into a 50ml Falcon tube containing ddH_2O. Select **Wash Injectors…** from the **Control** menu. Accept the default settings (30 injections) and click **OK**.
2. Repeat this wash step with 70%EtOH (30 injections). Replace each injector tube into the empty Falcon tubes and perform a final "Wash" that will just dry the injector tubing.

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