Rudnick 2011

Luciferase Assay Protocol-
Reading Luciferase and Renilla signal using Promega Dual Luciferase Reporter Assay

NOTE: in order to read luciferase using this system, cells must be previously transfected with a luciferase reporter construct (either purchased or cloned for gene of interest) and Renilla plasmid (in lab stock)

- **Reagents:**
  Dual Luciferase Reporter Assay System (Promega cat # E1910)
  Epindorfs
  Luminometer
  Sample plate(s)
  PBS
  Distilled water
  Cell scrapers

- **Preparation:**
  1. Thaw frozen buffers in 37 C waterbath.
  2. Turn on luminometer to warm up before taking readings.
  3. Dilute Passive Lysis Buffer (PLB) 1:5 in distilled water and mix well.
  4. Prepare Luciferase Assay Reagent II (LARII) by adding 10 ml Luciferase Assay Buffer II to lyophilized substrate. Aliquot LARII in epindorfs and store unused aliquots at -80 (stable for up to 1 yr) protected from light.
  5. Prepare an adequate volume of Stop & Glo Reagent to perform desired number of assays. Dilute Stop & Glo Substrate 1:50 with Stop & Glo Buffer in a 15 ml falcon tube.

  *100 ul Stop & Glo Reagent/assay * _____ assays (aka readings) = ____ ul Stop & Glo needed + _____ ul pipet error = _____ ul total volume of Stop & Glo Reagent needed*

  6. Predispense 100 ul of LARII into appropriate number of epindorfs to complete desired number of luciferase and Renilla readings.
  7. Check that luminometer is programmed to perform a 3 second premeasurement delay, followed by a 10 second measurement period for each assay. (NOTE: these are default settings on the Kuperwasser luminometer).

- **Protocol:**
  1. Aspirate media from cells and wash with PBS.
  2. Aspirate PBS and add appropriate amount of diluted PLB (see chart below) to plates (lysis).
  3. Lyse cells using a cell scraper.
  4. Transfer 20 ul of lysis sample to epindorf tube containing LARII, mix up and down with a pipet 2-3 times. Place tube in luminometer and take reading.
  5. If luminometer does not print, record the luciferase activity measurement by hand.
  6. Remove epindorf from machine and add 100 ul Stop & Glo Reagent.
  7. Vortex sample for a few seconds.
  8. Place in luminometer again and take Renilla reading. Record by hand if luminometer does not print.
  9. Discard this tube, and repeat again with next sample.
  10. Calculate Luciferase/Renilla signal intensity, and normalize signal to that from cells transfected with a control-luciferase reporter plasmid and Renilla.

Multiwell Plate	1X PLB
6-well culture plate	500µl
12-well culture plate	250µl
24-well culture plate	100µl
48-well culture plate	65µl
96-well culture plate	20µl