LUCIFERASE ASSAY:

1. After cell treatment, wash cells once with PBS. Add **lysis buffer**
   200 ul for 6-well plates, 125 ul for 12 well, or 75 ul per well in 24 well plates.

2. Scrape cells free from the well with a rubber policeman or tip of pipette and transfer to a microfuge tube and spin at highest speed for 5 min at RT.
3. Add 50 ul of the cell extract supernatent to 350 ul of freshly-prepared **assay buffer** in an assay tube (12 X 75 mm polypropylene).
4. Place the assay tube in a luminometer and inject with 100 ul of 0.5-1 mM luciferin.
5. Measure peak-integrated luminescence after a 5 sec delay over a 10 sec window.

LYSIS BUFFER

25 mM Tris/phosphate
4 mM EGTA
1% Triton X-100
10% glycerol
2 mM dithiothreitol

For 50 ml:

1.25 ml 1 M Trizma base, 39.25 ml QH2O, 4 ml 50 mM EGTA. Adjust pH to 7.8 with dilute o-phosphoric acid. Add 5 ml glycerol and 0.5 ml of Triton X-100. Stable if stored at room temperature briefly. Just prior to use 2 ull of 1 M dithiothreitol per 1ml to make complete buffer.

ASSAY BUFFER

25 mM Tris/phosphate
20 mM $MgSO_4$
4 mM EGTA
2 mM ATP
1 mM dithiothreitol

20 ml

0.5 ml Trizma base, 17.1 ml dH_2O, 0.6 ml 1 M $MgSO_4$, 1.6 ml 50 mM EGTA. Adjust pH to 7.8 with dilute o-phosphoric acid. Add 200 ul of 200 mM ATP and 20 ul 1 M dithiothreitol just before use.

1 mM D-LUCIFERIN

Directions for a 25 mg sample: Dissolve 0.17 gr dithiothreitol in 110 ml of QH20. Add 1 ml of this DTT solution to the 25 mg d-luciferin. Add 20 ul of 10N NaOH to dissolve the luciferin. Dilute this into the remaining 109 ml of DTT solution and store as 1.25 ml aliquots at –20C until use.

REAGENTS

d-luciferin Molecular Proges L2911
ATP Sigma A5394
Triton X-100 Sigma T6878
EGTA Sigma E3889