LUCIFERASE ASSAY

1. Remove media from cells, wash once with 1xPBS, add 1 ml ice cold PBS to cells, scrape and transfer to eppendorf tube. Spin 15 sec at full speed.

2. Resuspend pellet in 150 µl of 25 mM HEPES, pH 7.18, 15 mM $MgSO_4$, 1 mM DTT.

3. Freeze and thaw 3 times using dry ice and a 37°C waterbath. After the final thaw, spin at full speed for 5 min, save supernatant. Do BioRad protein assay.

4. Take 30 to 50 µg of each sample and add sample buffer to a volume of 175µl. Add 175 µl of assay buffer. The total volume should be 350 µl.

Sample Buffer
25 mM HEPES pH 7.8
15 mM $MgSO_4$

Assay Buffer
25 mM HEPES pH 7.8
15 mM $MgSO_4$
5 mM ATP
1 µg/ml BSA
(For 5 mls, add 50 µl stock ATP and 5 µl stock BSA)

5. Inject 100 µl of luciferin into each tube using luminometer injection port and take a ten second integrated light reading. Be sure luciferin is at room temperature before using. Use 1 µl of stock luciferase (1 ng) as a control. If everything is okay, a reading of 16,000,000 to 22,000,000 RLU should result.