PROTOCOL FOR LUCIFERASE ASSAY
Chris Song, 09/06/05

1. Reconstituting Luciferase Assay Substrate (lyophilized) with Luciferase Assay Buffer. It should be stored in aliquots and is stable at -70°C in the dark for up to 1 year after reconstitution or initial use.

2. Take out Luciferase Assay Substrate (in aliquots) out of -70°C freezer, put the bottle in cold water to thaw the Luciferase Assay Reagent at temperatures below 25°C for 30 min. Shake it gently to make it mixed well.

3. Add 4 volumes of water to 1 volume of 5X lysis buffer (stored at -20°C). Note: Cell Culture Lysis Reagent provides efficient lysis within minutes (5min). Reporter Lysis Buffer (RLB) is a mild lysis agent and requires a single freeze-thaw cycle to achieve complete cell lysis. For applications involving the co expression of firefly luciferase with a second reporter gene, we recommend preparing cell lysates with RLB. RLB has not been qualified for use with plant or bacterial cells.

4. Aspirate the medium of every well carefully and quickly. Add enough 1X lysis buffer to cover the cells. (Add 100ul lysis buffer per well of 24 wells plate). Rock culture dishes several times to ensure complete coverage of the cells with lysis buffer. Note: It can save time to use tips to disturb the buffer and cells. If using RLB, perform a single freeze-thaw to ensure complete lysis.

5. Put 20ul Luciferase Assay Substrate in BD Moonlight cuvettes (12×47 , for luminometer)

6. Power on the luminometer. Take Program three and perform a 5-second measurement delay followed by a 10-second measurement read for luciferase activity.

7. Add 50ul lysis cell suspension to cuvettes, Mix them well by quickly pipetting up and down twice.

8. Place the tube in the luminometer and initiate reading.(press “start”)

9. Record the reading.

10. Press “next”, and then test other samples as the steps ahead (step 7-9).