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# Luciferase Assay

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## Procedure

- Both LAR II and Stop&Glo are light sensitive and must be kept on ice when not in use.

1. Transfect 6 well plates of cells using lipo200 or lipo/plus protocol, using 5ng of Renilla vector as a transfection control with the experimental DNA.

2. 48 hours after transfection, lyse cells in 1x Passive Lysis Buffer (PLB, 5x stock provided by Promega Luciferase kit at –40) by either adding 350uL 1xPLB to plates and rock for 15 minutes, room temperature and collecting cells or by scraping or trypsinizing cells off of plastic and resuspending in 1xPLB.

3. Freeze/thaw the lysates 2x alternating between –80 and room temperature (it usually takes about 15 minutes to freeze)

4. Spin down the lysates 2 minutes full speed at room temperature.

5. Aliquot 20uL cleared lysate per well (x4 wells) into black luminometer plates.

6. Add 100uL LAR II reagent/well to read luciferase values, this is a time sensitive process so make sure to add just before putting the plate in the TopCount
   6.1. LAR II reagent is prepared by mixing 10mL Luciferase Assay Buffer II with Luciferase assay substrate. 10mL LAR II is sufficient for 1 full 96 well plate. This buffer can be stored for 1 year at –80.

7. After reading is finished, add 100uL Stop&Glo reagent (to read renilla values), this is a time sensitive process so make sure to add just before putting the plate in the TopCount
   7.1. Stop&Glo reagent is prepared by mixing 10mL Stop&Glo buffer with 200uL 50x Stop&Glo substrate. 10mL Stop&Glo reagent is sufficient for 1 full 96 well plate. This reagent must be made fresh every time.