

Dual Luciferase Assay

Lucho Fuentealba 2008

All substrates and solutions are provided by Dual-Luciferase Assay System from Promega (Cat# E1960)

Harvesting of cells

- Dilute the Passive Lysis Buffer (PLB)
- Wash wells one time with PBS. Do not wash 293T cells since they detach easily
- Place 200 μ l of 1X PLB in each well (for a 12-well plate)
- Rock the plate for 15 min at RT
- Take the extract and place in microfuge tubes. Keep at -20°C until use

Preparing the substrate

- Thaw on water the Luciferase Assay Buffer II (green label) and Stop & Glo Buffer (blue label)
- Pour the content of the Luciferase Assay Buffer bottle into the lyophilized Luciferase Assay Substrate vial. Resuspend up and down
- Take the needed amount (100 μ l per sample) of Stop & Glo Buffer and add 1/50 of Sto & Glo Substrate. Mix by vortexing

Preparing the sample

- Thaw microfuge tubes at RT. Centrifuge them for 2 minutes at 4°C at max speed
- Place 20 μ l of sample into a 96-welled plate (white, Nunc Cat# 236105)

Setting the fluorescent plate-reader

- Turn Fluorometer 20 min before use. Go to SoftMax Pro software.
- Go to "Protocols", then "Luminescence LMax II" and "Dual Luciferase"
- Press the "Setting" button in Plate 01 and choose:
 - Integration: 1000
 - Top read
 - Emission: Lm1 All
Lm2 All
- Automix: 3 sec
- Wells to read: Choose area of wells to read
- Plate: opaque.

Luciferase assay

- Add 100 μ l of mixed substrate into each well containing sample
- Put into the reader and press "Read". This step will give you the measurements for your specific reporter (Firefly luciferase)
- Once the machine has finished measuring luminescence and the plate is out, go to "new plate"
- Add 100 μ l of mixed Glo & Stop substrate into each well containing sample and substrate. Put back into the reader and press "Read". This step will give you the measurements for your transfection control reporter (Renilla luciferase)
- Divide Firefly-Luc values by Renilla-Luc values (FF/R) to obtain a corrected quantification for your reporter luminescence