PROMEGA BRIGHT-GLO LUCIFERASE ASSAY
(96 well plate)

Day 1: Plate cells at 7,500 cells/100 μl/well

Day 2: Treat cells as desired for appropriate time

Day 3: Assay for luciferase activity with Promega Bright-Glo reagent

Luciferase assay:

1. Thaw assay buffer in room temperature water.
2. Add assay buffer to lyophilized substrate.
   (dissolved substrate is stable for 1 mo. at –70°C)
3. Turn on luminometer and computer, open "Bright-Glo" program and edit template as desired.
4. Equilibrate cells to be tested to room temp (about 5 min).
5. Add 100 μl substrate to each well using multipipetter.
6. Allow cells to lyse for 5 min.
7. Transfer 200 μl lysed cells/substrate reagent to white opaque luminometer microtiter plate using multipipetter.
8. Read in luminometer (Molecular Devices)
   Bright Glo protocol:
   endpoint protocol
   Integrate:5 sec
   Preread: off
   No injections
   One 96 well plate will take about 10 min to read.

Comments:
The half life of the substrate is a little more than 25 min, so the assay should be processed quickly.

Reference: Promega Bright-Glo Luciferase Assay system

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