

## Luciferase Reporter Assay

Jan-11-2008, Hirotaka Watanabe

### ***Background***

Luciferase can emit luminescence during its enzymatic reaction. Using luciferase gene as a transcriptional reporter, we can determine the transcriptional activity of certain genes with high sensitivity. Promega incorporation sells several reporter plasmids in this method (now pGL4 series). As you know, however, Transient transfection step sometimes varies between experiments or wells. To normalize the transfection efficiency, we can use two different luciferase genes (*firefly* and *renilla*) in two distinct plasmids. Promega also sells the kit for this end.

### ***Reagent***

- ✓ Dual-Luciferase® Reporter Assay System 100 assay (Promega, cat# E1910), \$166.00

### ***Procedure***

#### Sample preparation

1. Prepare appropriate culture (such as fibroblast or primary neuron), and transfect the pGL4.10 derivative, which contains specific promoter or transcription binding sites upstream to firefly luciferase, and the pGL4.74 (tk-renilla luciferase).

*You should determine a suitable ratio of plasmid DNA beforehand. I usually transfect pGL4.10 and pGL4.74 plasmid at the ratio of 10:1 – 20:1.*

2. Some days after transfection, aspirate medium and wash with PBS (-).
3. After completely removing PBS, add appropriate amount of Passive Lysis Buffer, and rock thoroughly for 20 min at RT.
4. Collect the lysate into a new eppendorf tube, and measure the luminescence as follows.

*You can store these lysates at -20C for several weeks.*

#### Measurement

I use “Wallac 1420 (PerkinElmer)” as a luminometer. This equipment is in HIM-624. PUMP2 is now out of order, so we have to use two different substrates (LARII and STOP&Glo) sequentially if you want to apply Dual-luciferase assay.

1. Click [Victor2] icon on the desktop screen to start ‘Wallac 1420 Manager’.
2. Select Tools/Dispenser Maintenance. Select [Flush] to wash out the line leading to dispenser following the set of milliQ to Pump1. It washes automatically by 10 dispense cycles. Take the milliQ tube out of the line, and select [Fill] to get off water from the line.

3. Set the substrate tube to Pump1, and select [Fill] to fill the line with substrate. Close the window.

*To save the substrate, I usually set the substrate tube after 1<sup>st</sup> dispense cycle ([Fill] command executes total four dispense cycles).*

4. Select Tools/Start Wizard. Click [Next] after selecting the appropriate program (for my luciferase measurement, [Protocols]-[Users]-[Hiroataka]-[DualLuciferase1]).

*You can generate new protocol by selecting Tools/Explore- File/New protocol.*

5. Select the wells on screen, and click [Next]. Write down any text description (option), and click [Next]. Click [Finish] to start measurement of luminescence. You can see luminescence results on 'Live display' tab. Measurement of one plate (96 well) takes about 50 min.

6. Select Tools/Explore to show view of all the results on the right of screen. Click the desired result to display 'Result viewer'. Save the result (File/Export).

*You will obtain average luminescence values (CPS) in the chart.*

7. Select Tools/Dispenser Maintenance. Select [Empty] to reverse the substrate that remains in the line. Set milliQ tube to Pump1, and select [Flush]. Take the milliQ tube out of the line, and select [Fill] to get off water from the line.

8. If you want to do second substrate, you can repeat the step #3 to #7.