Use of NL-Luc Luciferase Reporter Virus

NL-Luc virus is a single cycle reporter virus that has been useful in a large number of experiments that study viral entry. The virus should be used in a P3 lab, although its potential biohazard is very low since it has frame shift in ENV and a large insertion of luciferase.

Reagents:
Luciferase assay kit from Promega containing lysis buffer and assay reagent.

PROTOCOL
For adherent cells

1. Plate out cells the day before infection in 24 well dishes at 2 X 10^4/well in a volume of about 0.5 mls.

2. The next day infect the cells with NL-Luc virus (10-40 ng p24)/well in a total volume of 200-300 μl with polybrene at a final concentration of 4 μg/ml. Let virus stay on cells 4 hrs-overnight). Remove medium and replace with 1 ml normal medium. Let cells grow for 3-4 days. Assay on day 4 gives higher activity than day 3, but for rapidly growing cells, day 3 may be more than sufficient.

3. On day of harvest, remove medium, wash once with 500 μl PBS, remove, add 150μl commercial luciferase lysis buffer. Wait about 2 mins. Pipette well up and down to lyse cells and put in eppendorf tube. Put supe in fresh eppendorf tube and freeze tubes until ready for assay.

4. To assay, thaw lysates. Microfuge 2 min. to pellet cell debris. Take 20μl of lysate and add 100 μl Promega luciferase assay buffer. Read in luminometer. Can refreeze the rest of the lysate for future use.

For nonadherent cells
Don't need to do anything a day ahead of time.

1. Pellet some cells to be infected. Resuspend in a small volume and count. Do infection in 24 well dish as above. We usually use more cells (about 5 X 10^4). Infect with same amounts of virus as above in a similar volume. Note, none of these parameters is critical: the virus infects well under many different conditions. After 4 hrs to overnight, add 0.5 mls medium (we try to save work by not washing out the virus: this seems to be fine).

2. After 3 or 4 days, make lysates by transferring cultures to eppendorf tubes, washing in 0.5 ml PBS, spinning at low speed to pellet the cells, removing the supe and adding 100 μl of commercial lysis buffer. Freeze lysates.

3. Thaw lysate. Microfuge 2 mins to remove debris. Assay 20μl portion of the lysate in a standard luciferase assay as above.