VIRAL PLAQUE ASSAY

Adapted from the Invitrogens' Bac-to-Bac manual

Materials:

- 30 ml of exponential culture of SF9 cells diluted to $5x10^5$ cells/ml
- 6-well plates (2 each)
- 1 bottle 4% Agarose Gel (Invitrogen cat. number SKU # 18300-012)
- 1 bottle SF-900 (1.3X) medium (Invitrogen cat number SKU #10967-032)
- 1 sterile bottle
- 0.5 ml baculovirus supernatant (preferably from transfection or 1st amplification)
- 100 ml ESF921 medium (Expression Systems; cat. # 96-001)

Procedure:

1. Under sterile conditions dispense 2 ml of cell suspension per well.

2. Allow cells to settle to bottom of plate and incubate, covered, at RT for 1h.

3. Place the bottle of agarose gel in the 70oC water bath. Place the empty bottle and the SF-900 (1.3X) in the 40oC bath.

4. Following 1h incubation of the plates at RT, observe monolayer under the inverted microscope to confirm cell attachment and 50% confluence.

5. Produce an eight-log serial dilution of the harvested viral supernatant by sequentially diluting 0.5 ml of the previous dilution in 4.5 ml of SFM medium in 12-ml disposable. You should conclude with 8 tubes containing each of a 10-1 to 10-8 dilution of the original virus stock.

6. Move the six well plates and the tubes of diluted virus to the hood. Label the plates, in columns of two, "10-3, 10-4, 10-5, 10-6, 10-7, 10-8".

7. Sequentially remove the supernatant from each well, discard, and immediately replace with 1 ml of the respective virus dilution to each duplicate well. Incubate for 1h at RT.

8. Move bottles from waterbaths to sterile hood when agarose has liquid (20 to 30 min). Quickly dispense 30 ml of the SF-900 (1.3X) medium and 10 ml of the 4% agarose gel to the empty bottle and mix gently. Return the bottle of plaquing overlay to the 40oC water bath until use.

9. Following this second 1h incubation, return the bottle of diluted agarose and the 6-well plates to the hood.

10. Sequentially (from high to low dilution) remove the virus from the wells and replace with 2 ml of the diluted agarose. Work quickly to avoid desiccation of the monolayer. A Pasteur pipette connected to a vacuum pump easily removes inoculum traces.

11. Allow gel to harden for 10 to 20 min before moving.

12. Incubate at 27°C in a humidified chamber in incubator for 4 to 10 days.

13. Recombinant virus produces milky/gray plaques of slight contrast visible without staining or other detection methods.

14. Monitor plates daily until the number of plaques counted does not change for two consecutive days.

15. To determine the titer of the inoculum employed, an optimal range to count is 3 to 20 plaques per well of a 6-well plate. The titer (pfu/ml) may be calculated by the following formula:

16. Optional: Overlay of agarose with Natural Red dye:
Prepare 0.5% agarose solution in SFM
Add Natural Red solution to a final concentration of 50mg/ml (dilute 3.33mg/ml stock 1:66.6).
Overlay 1ml of the dye solution to each well (on a 6 well plate).

Pfu/ml (of original stock) = 1/dilution factor x number of plaques x 1/(ml of inoculum/plate)