

## Viral Plaque Assay: Abridged

### Materials:

- 30 ml of exponential culture of SF9 cells diluted to  $5 \times 10^5$  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 1<sup>st</sup> amplification)
- 100 ml ESF921 medium (Expression Systems; cat. # 96-001)

1. Plate 2 x 6 wells with  $1 \times 10^6$  cells per each well and incubate for 30-60min.
2. Serial dilute 0.5ml of the original virus stock into 8 tubes with 4.5ml medium each
3. Aspirate the medium from the cells and add 1ml from dilutions -3 to -8 to 2 wells
4. Incubate at room temp. for 1h (you can rotate very slowly on a "belly dancer") covered with aluminum foil.
5. Incubate 4% agarose in 70°C and 21ml of SF900 x 1.3 in 50ml tube in 40°C bath
6. Nuc the agarose in a microwave oven for a few seconds till it boils (just to make sure it is all dissolved), and add 7ml to the 50ml tube containing the SF900x1.3
7. Aspirate the medium COMPLETELY from each duplicate and overlay 2 ml of the diluted agarose. (quickly, before the cells dry out!)
8. Leave at RT form 20 min to let agarose solidify. (covered with aluminum foil)
9. Place in humidified chamber in the incubator for at least 5 days. (plaques can be seen well usually after 7 days, and can be picked up after about 10 days)

Pfu/ml (of original stock) =  $1/\text{dilution factor} \times \text{number of plaques} \times 1/(\text{ml of inoculum/plate})$

