



Titering of Baculovirus by Plaque Assay

1. Seed 4 ml of log phase cells (>98% viable) at 5×10^5 cells per ml. (2×10^6 viable cells per plate into Nunc Delta 60 x 15 mm tissue culture dish.) Rock plates to distribute cells evenly and allow cells to attach for at least 1 hour. Examine cells from several plates under an inverted microscope to confirm cell attachment. The cells will not be confluent but will grow and divide over the course of the plaque assay.
2. Prepare 10 fold dilution of virus inoculum in the range of the expected titer allowing 1 ml of diluted virus for each plate. Dilute virus in sterile ESF 921 medium. Each dilution should be tested in triplicate.
3. After cells have attached in step 1, remove media overlay and add 1 ml of diluted virus inoculum to each plate.
4. Rock plates (keep in dark) at room temperature or at 27°C for one hour to evenly distribute virus.
5. While plate are incubating, prepare agarose overlay as follows:
 - 5.1 Prepare 4% agarose by suspending 2 grams of low melting point (Sea-Plaque) agarose in 50 mls distilled water in a small bottle and autoclaving for 15-20 minutes. Agarose may be prepared in advance and then melted in a microwave prior to use. Equilibrate melted agarose to 37°C in a waterbath.
 - 5.2 Pre-warm ESF 921 medium to 37°C .
 - 5.3 Immediately prior to use, microwave 4% sea-plaque agarose to melt. Mix 3 parts ESF 921 media to one part agarose (3:1 ratio) and store 30 to 40°C until ready to use.
6. At the end of the one hour incubation period in step 4, use Pasteur pipettes to remove all virus overlay from each plate.
7. Slowly add 4 ml of medium:agarose overlay to the edge of each plate. Rock each plate immediately after adding overlay to spread agarose evenly. Leave plates undisturbed for at least 1 hour to allow overlay to solidify.
8. Incubate plates in a humid environment at 27°C for 6 days. If a 27°C incubator with 80-100% humidity is not available, seal the plates in a zip lock bag and add damp towels to provide humidity.
9. Plaques can be observed by holding plates at an angle (approx 45°) or by placing over a dark background. Plaque purified virus can be obtained by picking plaques from plates containing 50 or fewer plaques (see support protocol below). If plaques are difficult to visualize and not needed for purification, the plates can be stained with either MTT or Neutral Red.
10. MTT Stain: Stain the plates by overlying with 2 ml of MTT (Sigma): ESF 921 medium: 4% agarose preparation and store plates in the dark. Plaques will appear as clear areas in a blue background after about 3 hours. Staining may require overnight.

To prepare 20 ml of MTT (Sigma): ESF 921 medium: 4% agarose (Prepare immediately prior to use).

ESF 921 Medium	14.36 ml
MTT stock	0.64 ml*
4% Sea-plaque agarose	5.0 ml



* MTT stock = 5 mg/ml in sterile distilled water. Prepare in glass bottle and protect 0.5% MTT stock from light

11. Neutral Red Stain:

Plaque Purification Protocol

1. Mark plaques on bottom of plate. Using a sterile 1 ml pipette tip, remove agarose plug from marked area. Pick at least 5-10 plaques.
2. Inoculate 1 ml of ESF 921 in a 1.5 ml sterile eppendorf tube with agarose plug. Rotate for overnight to elute virus.
3. Seed 12 well plate with 5×10^5 Sf9 cells in 2 ml ESF 921. Transfer 200 ul of each eluted plaque to a well of the 12 well plate. Incubate 3-5 days in humidified incubator.
4. Collect supernatant and examine for presence of gene of interest by PCR or expression.