THERION
BIOLOGICS

Tissue Culture Department

VACCINIA VIRUS : CELL CULTURE INFECTIONS

- Determine the number of cells in your culture vessel.
- Infect the cells at m.o.i.= 0.1 PFU/cell in D-MEM supplemented with 2 % FCS (antibiotics are optional). Use the minimal volume that would cover the cells. Distribute the inoculum uniformly on the monolayer and incubate at 37 °C and 5 % CO_2.
- After 30 min., aspirate the inoculum, replace with D-MEM 2% FCS and bring back to the incubator.
- Monitor the progress of infection under the microscope after 24 hs. At this m.o.i. the virus will be ready to harvest at 48 hs aproximately.
- To harvest the cell-associated virus, discard the supernatant of infection and resuspend the infected cells in 1 mM Tris pH 9.0.
- Perform three cycles of freeze-thaw.
- Purify through sucrose cushion (see protocol).
- Determine the viral titer (see protocol).

THERION
BIOLOGICS

Tissue Culture Department

VACCINIA VIRUS: SUCROSE CUSHION PURIFICATION

Procedure:

1. Preparation of the viral sample

- Freeze / thaw virus three times, vortex vigorously and spin down for 10 min at 1.2 K.
- Collect and save the supernatant.
- Resuspend the pellet by vortexing in 15 ml of 1 mM Tris pH 9.0.
- Centrifuge for 10 min at 1.2 K. Pool both supernatants.

2. Sucrose cushion

- Add 15 ml of 36% (w/v) sucrose in 1 mM Tris pH 9.0 into an ultraclear centrifuge tube.
- Slowly, layer 20 ml of viral sample on top of the sucrose cushion. Make sure that the sucrose and viral solutions do not mix.

3. Ultracentrifugation

- Place centrifuge tube in SW28 rotor buckets. Weigh the buckets and balance by adding 1 mM Tris pH 9.0 if necessary.
- Centrifuge at 20 K for 1 hour at 4 °C.

4. Pellet resuspension

- Immediately remove the tubes from the buckets at the completion of the spin. Aspirate the supernatant, invert the tube on a paper towel and allow to drain taking care not to disturb the pellet.
- Resuspend the viral pellet with a Pasteur pipet in 1 ml of 1 mM Tris pH 9.0. Wash the tube using a small volume of 1 mM Tris pH 9.0 and add the wash liquids to the resuspended viral preparation.

Note: Sucrose purified virus is ready for titration (see protocol).

Freeze at -80 ° until ready to use.

THERION
BIOLOGICS

Tissue Culture Department

VACCINIA VIRUS: PLAQUE ASSAY

Procedure:

- Mix 10 µl of vaccinia virus stock with 10 µl of sterile 0.25 mg/ml trypsin. Vortex vigorously.
- Incubate at 37 °C in waterbath, vortexing at 10 min intervals during the incubation.
- Immediately after, prepare a 10^{-2} dilution by adding 0.98 ml of DME, supplemented with 2% FCS and antibiotics. Continue serial dilutions to 10^{-10}, vortexing carefully and changing pipet tips after each dilution.
- Aspirate media from cells in 6-well plates.
- In duplicate, add 200 µl of virus dilution and 1 ml of DME 2% FCS plus antibiotics. Distribute the inocula well over the monolayers.
- Incubate 30 min at 37 °C, 5% CO2.
- Aspirate the inocula from plates.
- Add 3 ml of DME supplemented with 2% FCS and antibiotics.
- Incubate at 37 °C and 5 % CO2 for 48 hs.

Staining:

- Aspirate media.
- Add 2 ml of crystal violet (0.1% crystal violet in 20% ethanol) stain per well.
- Leave at room temperature for 10 min.
- Aspirate the stain, rinse by submerging in H20 and allow to dry.
- Count plaques and determine the viral titer.