Baculovirus Plaque Assay in 6-Well Plates

Modified from Bac-N-Blue™ Transfection and Expression Guide from Invitrogen.

Materials Needed:

Sf9 cells growing in Sf900 II SFM
1.3X Sf900II SFM (Invitrogen)
SeaPlaque Agarose (Lonza)
6- well Tissue culture plate
42°C water bath
10 mL sereological pipettes
Tissue Culture Hood
26°C incubator
Sterile 1x PBS
tetrasodium thiazolal blue bromide (MTT)

Day 1:

Make 100 mL of 4% SeaPlaque Agarose

4 g	SeaPlaque Agarose
100 mL	MilliQ water

Autoclave 20 min at 121°C to sterilize.

Morning of Day 2:

1. Microwave the 4% SeaPlaque Agarose and place in 42°C water bath along with a bottle of 1.3X Sf900II SFM . Allow to equilibrate to ~42°C (approximately 1-2 hrs).

2. During the time of equilibration count your Sf9 cells and make sure the cells are actively dividing and have a viability of 95% or greater.

3. Label the lids of the 6 well plates with the appropriate dilution and make sure to have duplicate wells for each dilution to be tested and include a negative (no virus) control.

Put 2 mL of Sf900II SFM into each well of the 6-well plates to be used. Seed $1x10^6$ to $1.2 x10^6$ cells to each well. Gently rock the plate from side to side then let cells adhere to the 6 well plate for 0.5 to 1 hr.

4. While cells are adhering make 3 mL serial diluitions for each virus to be tested. (I make 10^{-2} to 10^{-7} dilutions and do the plaque assay on dilutions of 10^{-5}, 10^{-6}, and10^{-7} and do the assay in duplicate.)

5. After dilutions are made. Remove the media off the cells in the 6 well plate and then put 1 mL of control media or the appropriate virus dilution in each well. Gently rock the plate back and forth 3 times and then let sit in the tissue culture hood for 1 hour.

6. 10 minutes before the hour incubation is up from step 5. Make the 1% SF900II agarose solution (see below). Make sure to make enough to put 2mL of 1% SF900II agarose solution on each well to be assayed.

1% Sf900II SFM agarose solution
Make under sterile conditions

37.5 mL of 1.3X SF900II SFM
12.5 mL of 4% SeaPlaque agarose

Put at 42^{o}C and remove each tube and surface sterile 5 minutes prior to use.

7. Remove media from the wells to be assayed. Place 2 mL of ~40^{o}C 1% Sf900II SFM agarose solution over each well. Allow agarose to solidify (~ 20 minutes). After this time wrap parafilm around the plates and place in a zip lock bag with a paper towel dampened with 5mM EDTA and place at 26^{o}C in the dark.

8. Plaques should form around 5-7 days after infection.

Visualisation of Plaques with tetrasodium thiazolal blue bromide (MTT)

Plaques can be seen visually without this dye, but for easier visualization the plaques can be "developed" with MTT.

1. On the morning of visualization of plaques make a 0.1mg/mL MTT solution in 1xPBS (You will be using 300 mL of the 0.1mg/mL MTT solution per well). The solution will be yellow.

2. Remove plates from the zip lock bag and take off the parafilm. Then place 300 mL of the 0.1mg/mL MTT solution per well. Place the plates at room temperature in the dark for 3 to 5 hrs. The MTT will turn purple where the cells are alive and you will be able to see the plaques and count them.

2. Count the plaques in each duplicate viral dilution well and take an average and then calculate the number of plaque forming units/mL.

(1/dilution)xnumber of plaques = pfu/mL

To compute the amount of virus you need for an infection for the desired multiplicity of infection (M.O.I.)

$$\frac{\{\text{M.O.I.(pfu/mL)x number of cells}\}}{\text{titer of virus (pfu/mL)}} = \text{mL of inoculum needed}$$