kempbio

Baculovirus Plaque Assay Protocol

Required Materials:
SF9 cells
Media for SF9 cells: We use SF900III from ThermoFisher Cat# 12658-027
Sf900 1.3X: ThermoFisher Cat#10967-032
FBS: Source not important, just make sure it is not toxic
Agarose, low gelling temperature: Sigma Cat# A9045
6 well dishes

Prepare a 4% w/v solution of Agarose and autoclave to sterilize
Keep the agarose in a 74°C water bath until use
Add 10% FBS to Sf900 1.3X and keep in a 37°C water bath until use

Dilute SF9 cells to a concentration of 5x10e5 cells/mL and seed 2mL well in 2 x 6 well dishes.
Allow the cells to attach for approximately 30 minutes.
While cells are attaching, make 1:10 serial dilutions of virus in media + 10% FBS (the range I usually do is 10^{-1} to 10^{-7})
After 30 minutes, gently remove media from wells.
Add 1mL of diluted virus or media + 10% FBS (as negative control) per well.
My plate layouts are:

Neg. Control	10^{-7}	10^{-6}
10^{-7}	10^{-7}	10^{-6}
10^{-6}	10^{-5}	10^{-5}
10^{-6}	10^{-5}	10^{-5}

Allow virus to absorb for approximately 2 hours (longer is fine).

Mix 3 parts Sf900 1.3X to 1 part Agarose in a 50mL conical.
For 2 plates, I use 22.5mL Sf900 1.3X and 7.5mL Agarose.
This mixture needs to stay warm enough to not solidify, but needs to cool enough not to kill your cells.
I invert the tube several times to start to cool it.
When the mixture is no longer hot (tested on the inside of your wrist) or measures about 30-31°C using an IR temperature gun, remove the virus from the plates and gently add 2mL/well agarose mixture.
Allow to solidify in the hood for 10-15 minutes.
Put plates into a plastic bag with a lint free towel moistened with a few drops of EDTA solution and incubate at 27°C for 6-7 days.
Add 0.5mL/well Neutral Red Stain for 15 minutes. Dump off excess. Count the plaques the next day.

5119 Pegasus Ct. Suite P Frederick, MD 21704
Phone: 240-629-8924
chris.kemp@kempbioinc.com