Adenovirus General Protocol for Infecting Cells

1.0 Purpose

1.1 Provide instructions on infecting cell lines with adenovirus vectors.

2.0 Materials

2.1 Equipment

Tissue Culture Hood
37°C Incubator w/ 5% CO_2

2.2 Supplies

1, 6-well Tissue Culture Plate
Sufficient volume to obtain 50 MOI of virus/well
Cell media

3.0 Procedure

3.1 Day Before Infection

3.1.1 Prepare a 6 well plate to be about 50% confluent the next day.
3.1.2 A general rule to follow when splitting cells is to note that an entire 6 well plate is about ¾ the surface area of a T-75, about ¼ the surface area of a T-150, and about 1/9 the surface area of a 10cm plate.

3.2 Infection Method 1: Infection Overnight

3.2.1 BEFORE BEGINNING, make sure the cells on the 6 well plate are about 50% confluent (if the cells are not ready, then you should not go any further).
3.2.2 Thaw your virus on ice *(it is important to store your virus at –80°C when you are not using it… anything higher than that and you may be losing titer!).*
3.2.3 Add a specific MOI of virus to each well according to your chosen dose curve. MOI means Multiplicity Of Infection, so in a well that contains 50 MOI, there are 50 viral genomes for every cell.
3.2.4 A typical dose cure (what we usually do) is 0, 50, 100, 200, 500, 1000 MOI. You do not need to change the media before doing this; you just simply add the virus to the wells already containing media. Also, it is important to have 0 MOI as a negative control to check your cell growth and death.
3.2.5 For figuring out the amount of virus you need to add for a certain MOI, use the formula: #cells * desired MOI= total PFU (*or Plaque Forming Units*) needed. Then use the formula: (total PFU needed) / (PFU/ml) = total ml of virus needed to reach your desired dose.

3.2.6 For example: You have a virus with a titer of $1.3x10^{11}$ PFU/ml and a well that contains $1.8x10^{6}$ cells. You want to make that well contain 200 MOI. Therefore, formula 1: ($1.8x10^{6}$ cells) * (200 MOI) = $3.6x10^{8}$ PFU desired. Then, formula 2: ($3.6x10^{8}$ PFU desired) / ($1.3x10^{11}$ PFU/ml) = 0.0028 ml or 2.8µl. Add the 2.8µl to the well and label it 200 MOI.

3.2.7 *Note: you may need to dilute your virus 1:10 or more.*

3.2.8 Add the appropriate amount of virus to each dose well, and label the wells with the correct MOI.

3.2.9 Place virus-infected cells in 37°C incubator until the next day (24 hours).

3.2.10 Replace media after 24 hours.

3.3 Infection Method 2: One Hour Dose Curve

3.3.1 BEFORE BEGINNING, make sure the cells on the 6 well plate are about 50% confluent (if the cells are not ready, then you should not go any further).

3.3.2 Thaw your virus on ice *(it is important to store your virus at –80°C when you are not using it… anything higher than that and you may be losing titer!).*

3.3.3 Prepare serial dilutions of virus in microfuge tubes using the same media that the cells are grown in.

3.3.4 Each well of the 6 well plate will get a total of 350µl of fluid. For figuring out the amount of virus you need to add for a certain MOI, use the formula: #cells * desired MOI= total PFU (*or Plaque Forming Units*) needed. Then use the formula: (total PFU needed) / (PFU/ml) = total ml of virus needed to reach your desired dose.

3.3.5 For example: You have a virus with a titer of $1.3x10^{11}$ PFU/ml and a well that contains $1.8x10^{6}$ cells. You want to incubate that well with 200 MOI. Therefore, formula 1: ($1.8x10^{6}$ cells) * (200 MOI) = $3.6x10^{8}$ PFU desired. Then, formula 2: ($3.6x10^{8}$ PFU desired) / ($1.3x10^{11}$ PFU/ml) = 0.0028 ml or 2.8µl. Add 348µl media and the 2.8µl of virus to a tube and label it 200 MOI. *Note: you may need to dilute your virus 1:10.*

3.3.6 Below is an example of microfuge tubes labeled A-F which follow a typical dose curve (0, 50, 100, 200, 500, 1000 MOI).

Example of 1 Hour Dose Curve Using a Virus With a Titer of $1.3x10^{11}$ PFU/ml and a 6 Well Plate Containing $1.8x10^{6}$ cells/well

Tube	Virus needed	Volume media	MOI
A	0 µl	350µl	0
B	0.7 µl	350µl	50
C	1.4 µl	348µl	100

D	2.8 µl	347µl	200
E	6.9 µl	343µl	500
F	14 µl	336µl	1000

3.3.7 Aspirate growth media from the wells.
3.3.8 Add the 350ml infection media to each well and rock the plate to make sure the cells do not dry out.
3.3.9 Place virus-infected cells in 37°C incubator, and rock the plate every 10-15 minutes for 1 hour.
3.3.10 Aspirate the viral media from the cells (into a vacuum flask containing bleach)
3.3.11 Add 2 mls of growth media (whatever you grew the cells in originally) to each well.
3.3.12 Place cells in 37°C incubator until the next day (24 hours).