

3. Puromycin kill curve

Optimization of puromycin selection condition

To generate a fully transduced population of cells it is important to determine the minimum amount of puromycin required to eliminate non-transduced cells. This is accomplished by performing a kill curve to determine the optimal puromycin concentration needed to eliminate non-transduced cells. A kill curve is a dose-response experiment where the cells are subjected to increasing amounts of antibiotic to determine the minimum antibiotic concentration needed to kill all the cells over the course of 2 to 7 days. For puromycin, the optimal concentration is the lowest concentration that kills 100% of non-transduced cells and shows maximal survival of transduced cells in 48-72 hours. The optimal concentration is cell type dependent. Performing a kill curve is recommended with each new cell type or when a new selection antibiotic or different lot of selection antibiotic is used.

Puromycin Kill Curve

1. Plate cells at appropriate density in 100ul complete growth medium per well in a 96-well clear bottom tissue culture plate. The recommended cell density for most cell types is 30-50% confluence at the day of transduction. Incubate at 37°C overnight.
2. Replace media with 45ul media containing 4ug-8ug/ml polybrene(depend on cell type).
3. Infect the cells with shRNA control virus (C6-4) at MOI 2 and MOI 6:

Virus MOI												
A												
B												
C												
D												
E		2	2	2	2	2	2	2	2	2	2	
F		2	2	2	2	2	2	2	2	2	2	
G		6	6	6	6	6	6	6	6	6	6	
H		6	6	6	6	6	6	6	6	6	6	

4. Spin the plates at 1100g at 30°C for 15-30min. Incubate at 37°C overnight.
5. Replace the media from the infected cell plates and add 100ul media containing various puromycin concentrations as shown below. Incubate at 37°C for 48-72 hours.

Puromycin concentration, ug/mL												
A		0	0.5	1	2	3	4	5	6	7	8	
B		0	0.5	1	2	3	4	5	6	7	8	
C		0	0.5	1	2	3	4	5	6	7	8	
D		0	0.5	1	2	3	4	5	6	7	8	
E		0	0.5	1	2	3	4	5	6	7	8	
F		0	0.5	1	2	3	4	5	6	7	8	
G		0	0.5	1	2	3	4	5	6	7	8	
H		0	0.5	1	2	3	4	5	6	7	8	

6. Remove the puromycin containing media from the cell plates.
7. Add 100ul of phenol red free DMEM medium containing 10% MTS. Incubate at 37°C for 0.5-3hrs.
8. Record the absorbance at 490 nm using a 96-well plate reader.
9. Determine the optimal puromycin concentration by choosing the minimum concentration of puromycin that causes complete cell death in non-transduced cells and maximal viability in transduced cells.