The RNAi Core

Version 2 (12/11/2014)

Polybrene Toxicity Test

Polybrene (Hexadimethrine bromide) is a polycation that neutralizes charge interactions to increase binding between the pseudoviral envelope and the cellular membrane. The optimal concentration of polybrene depends on cell type and may need to be empirically determined. Excessive exposure to polybrene can be toxic to some cells.

Check toxicity of polybrene

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. Incubate at 37℃ overnight.

2. Replace media with 50ul media containing 0-40ug/ml polybrene as table below .

Polybrene concentration, ug/mL												
A		**0**	**1**	**2**	**4**	**8**	**10**	**12**	**20**	**30**	**40**	
B		**0**	**1**	**2**	**4**	**8**	**10**	**12**	**20**	**30**	**40**	
C		**0**	**1**	**2**	**4**	**8**	**10**	**12**	**20**	**30**	**40**	
D		**0**	**1**	**2**	**4**	**8**	**10**	**12**	**20**	**30**	**40**	
E(MOI2)		**0**	**1**	**2**	**4**	**8**	**10**	**12**	**20**	**30**	**40**	
F(MOI2)		**0**	**1**	**2**	**4**	**8**	**10**	**12**	**20**	**30**	**40**	
G(MOI6)		**0**	**1**	**2**	**4**	**8**	**10**	**12**	**20**	**30**	**40**	
H(MOI6)		**0**	**1**	**2**	**4**	**8**	**10**	**12**	**20**	**30**	**40**	

3. Infect the cells with shRNA control virus (C6-4) at MOI 2 and MOI 6 in certain wells as below.

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. Incubate cell plate at 37℃for 24 hours, If the polybrene is toxic, test shorter exposure times (minimum ~2 hours).

5. Replace medium with complete culture medium for untransduced cell and complete culture medium containing puromycin (concentrations depend on cell type) for transduced cell (MOI 2 &6).

6. Grow cells at 37℃ for an additional 48 hours then check toxicity by counting viable cells.

7. Remove medium, add 100ul of phenol red free DMEM medium containing 10% MTS. Incubate at 37℃ for 0.5-3hrs.

8. Record the absorbance at 490 nm using a 96-well plate reader.

9. Determine the optimal polybrene concentration by choosing the concentration that results in lowest cell toxicity compared to no polybrene adding cell and maximal viability in transduced cells.