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GFP-VSV Infection V.1

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ABSTRACT

GFP-VSV Infection

PROTOCOL CITATION

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MATERIALS TEXT

-Aliquot of GFP-VSV virus (from -80°C)
-Serological pipets
-1.5 mL Eppendorf tubes
-Serum free DMEM media (high glucose) [depending on cell type]
-DMEM media (high glucose + 10% FBS) [depending on cell type]
-Ice bucket

1 If infecting transfected cells, infect them 24 hours after transfection.

2 Remove cells from 37 °C incubator.

3 Replace media with fresh media (with FBS) prior to infection with GFP-VSV.

4 Thaw VSV-GFP stock aliquot on ice

5 Dilute VSV-GFP in serum-free culture media. Perform the necessary calculations to determine the dilution that is required to obtain the desired MOI (multiplicity of infection).
Note: MOI is the number of viruses/number of cells. For example, a MOI of 1 = 500,000 PFU (plaque forming units) of virus per 500,000 cells.
Note: We typically start with a dilution range of MOI = 0.01, 0.1, 1 for optimization

6 Infect cells by dropping the diluted virus directly into the wells making sure to disperse the virus around the well.

7 Gently swirl the plate to help disperse the virus.

8 Leave the plate for approximately 24 hours in a 37°C CO_2 incubator. Then proceed with endpoint analysis.

Appendix - Sample Calculation

9 *Sample calculation*:
(MO1 = 1; Virus titre = $8.4x10^8$ virus/mL ; 500,000 cells/well;)
Step 1: calculate amount of PFU per well

Step 2: calculate how much to dilute the viral stock
$C_1V_1=C_2V_2$
Where V_1 = volume/well you have
C_1 = virus/mL calculated in step 1

C_2 = virus titre (stock concentration)

V_2 = x (unknown volume to be calculated)

You will add x mL of your virus stock per well. This volume will be low, and thus must be diluted in media for easier pipetting.