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Infecting Cells with SeV or RSV in A549 or LLCMK2 cells V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

Infection of SeV or RSV in A549 or LLCMK2 cells

Materials

INFECTION MEDIUM – SeV: Filter through 0.2 µm filter

	Component	Amount	Conc. Supp.	Product information
	DMEM	500 mL		Gibco Cat. # 11965092 (#11965118-cs)
	Pen/Strep	5.0 mL	100 U/mL Pen and 100 µg/mL Strep	Gibco Cat # 15140-122 – 10,000 U/mL
	BSA 35%	5.0 mL	0.35%	Sigma Aldrich Cat #A7979 – 35%
	Sodium Bicarbonate (NaHCO3) 7.5%	12 mL	0.18%	Gibco Cat #25080094 – 7.5%

TRYPSIN – TPCK (Worthington Biochemicals code: TRLVMF cat.no. LS004454)

INFECTION MEDIUM – RSV: Filter through 0.2 µm filter

	Component	Amount	Conc. Supp.	Product information
	DMEM	500 mL		Gibco Cat. # 11965092 (#11965118-cs)
	Gentamicin	500 µL	50µg/mL	Gibco Cat #15750060 (#15750078-pk) – 50 mg/mL
	Sodium Pyruvate	5.0 mL	1mM	Corning Cat #25-000-CI – 100mM
	L-Glutamine	5.5 mL	2 mM	Sigma Aldrich Cat #G7513 – 200mM
	FBS	10 mL	2%	

Infecting Cells with SeV or RSV in A549 or LLCMK2 cells

1 **Infecting cells with SeV or RSV in A549 or LLCMK2 cells**

1. Prepare a virus dilution in infection media with the correct virus-specific media (see materials section for media composition).
   - Calculate the volume of virus needed (X) using the MOI formula: (MOI*cell number)/Virus titer = X
   - The amount of infection media used to prepare the virus will depend on the size of the well or plate used - it is best to use the minimal volume needed to cover the well/plate to help ensure virus particle attachment to cells
2. Remove TCM
3. Wash cells twice with 1X PBS
4. Add XµL of virus suspended in infection media to each well (calculated in step 1)
5. Incubate the plate for 1 hr @ 37°C, rocking the plate/flask every 15 minutes
6. Remove the media
7. Wash twice with 1X PBS
8. Add infection media to each well/flask
   - For SeV infection: add SeV-Infection media, for SeV infection in LLCMK2 cells: add TRYPSIN –TPCK to the infection media for a final concentration of 2µg/mL TRYPSIN –TPCK
   - For RSV infection: add RSV-Infection media (2%FBS)
9. Place the plate/flask back in a 37ºC, 5% CO_2 incubator