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Version 1

SeV Titration in LLCMK2 Cells V.1

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Carolina Lopez¹

¹Washington University



Carolina Lopez

Washington University

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for titration of SeV

Materials

REAGENT	SOURCE	IDENTIFIER
Antibodies		
Chemicals and Recombinant proteins		
Sodium Pyruvate Solution 100 mM	Invitrogen	Cat#11360070
L-glutamine 200mM	Invitrogen	Cat#25030-081
Gentamicin	ThermoFisher	Cat#15750-078
Penicillin/Streptomycin	Invitrogen	Cat#15140122
NaHCO ₃	Sigma-Aldrich	Cat#S5761
Enzymes and other reagents		
DMEM	Gibco Life Technologies	Cat#11995073
Fetal Bovine Serum	Serum Source International	Cat#FB22-500
Bovine Serum Albumin 35%	Sigma-Aldrich	Cat#A7979
Trypsin 0.5%	Invitrogen	Cat#25300054
TPCK treated Trypsin	Worthington Biochemicals	Cat# LS004454
Critical Commercial Assays		
Experimental Model: Cell lines and viruses		
LLCMK2 Cells	ATCC	Cat#CCL-7
Chicken Red Blood cells	Lampire Biological Laboratories	Cat#7241409
Sequence-Based Reagents		
Software and Algorithms		

- Chicken Red Blood Cells Packed 5%: Innovative Research, Cat. ICHRBC5P15ML. (<https://www.innov-research.com/products/chicken-red-blood-cells-packed-5p?variant=21514823139408>)



Day 0

- 1 The day before titration prepare 96 well plates with LLCMK2 cells. Seed 2×10^4 cells in  100 μL of Tissue Culture Media (10% fetal calf serum, heat inactivated, endotoxin level 0.25 EU/mL; Hyclone, Logan, UT),  1 millimolar (mM) sodium pyruvate,  2 millimolar (mM) L-glutamine, and  50 mg/mL gentamicin) per well.
 - Dilute 2×10^6 cells/plate in  10 mL TCM. Dilute cells to proper concentration then put cell mix in tray and use multichannel to seed cells. Add  100 μL of cell mix/well.

Day 1

3d 1h

- 2 1. The day of the titration the cells should be 80-90% confluent.

3d 1h

 2. Prepare 1/10 dilutions of the virus in triplicates in Infection Media (DMEM + 50 mg/mL gentamicin + 35% BSA (Immuno, MP Biomedicals) + 5% NaHCO_3).
 - Use a different 96 well plate ("dilution plate")
 - Add  180 μL Infection Media to each well.
 - Add  20 μL virus to first well. Move  20 μL from each well down, changing tips every time.
 - Use a final volume of 200 μL /well for the dilutions. Take out 20 μL of last well.
 - CHANGE TIPS IN EVERY DILUTION.
 3. Dump the media of the plate containing the cells and WASH the FBS from the cells by adding/eliminating PBS to the wells. Repeat 2 times. Be careful not to scratch the monolayer.
 4. Add  25 μL from the virus dilutions to each well
 - Go from highest dilution to lowest dilution.
 5. Incubate for  01:00:00 at  37 $^{\circ}\text{C}$, 7% CO_2 .
 6. Add  175 μL of Infection Media containing 2 $\mu\text{g}/\text{mL}$ TPCK TRYPSIN (Worthington Biochemicals) per well (MAKE SURE YOU DON'T TOUCH THE WELLS WITH THE TIPS).
 - To make 2 $\mu\text{g}/\text{mL}$: 10 mL Infection Media + 10 μL TPCK.
 7. Incubate  72:00:00 .



Day 4

35m

3

1. Wash chicken blood.

- Add  5 mL chicken blood and  45 mL cold PBS and mix gently.
- Centrifuge  00:05:00 with 5 acceleration/5 deceleration, 1000 rpm.
- Discard supernatant.
- Repeat until supernatant is clear.

35m

OBS.: If using 5% Ready-to-use RBCs (*Chicken Red Blood Cells Packed 5%, Innovative Research, Cat. ICHRBC5P15ML*), usually one wash is enough since the RBCs come already washed and diluted to 5%.

- The resulting concentration of chicken RBCs prepared this way is 0.5%.

2. To determine virus titer by hemagglutination of chicken RBCs- to a round bottom 96 well plate add:

-  25 μ L PBS
-  25 μ L media from each well of infected plate.
-  50 μ L chicken blood

3. Agitate plate to mix.

4. Leave plate undisturbed in hood at RT for at least  00:30:00 to allow hemagglutination to occur

5. Score the titer by determining the last dilution with a positive HA. From the triplicates calculate the Tissue Infectious Dose₅₀ / 25 μ L.

Note

X = row in which last hemagglutination occurs
+ = no red dot (presence of virus)



HA of last row with virus	Dilution of last row with virus	TCID ₅₀
“ + + +”	10 ^{-X}	10 ^{X+0.7}
“+ + - ”	10 ^{-X}	10 ^{X+0.4}
“+ - -”	10 ^{-X}	10 ^{X-0.1}

Use spreadsheet to calculate titers and HA to infectivity ratios.

Protocol references

Modified for readability by Emmanuelle