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SeV fluorescent reporter virus titration by TCID50

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We use this protocol and it's working

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Abstract

Protocol to titer rSeV carrying a fluorescent reporter

Materials

1. Cultured LLCMK2 cells (ATCC, #CCL-7)
2. Tissue culture media and Infection media (See media prepare protocol)
3. 0.05% Trypsin (Thermo, #25300054)
4. PBS (Phosphate-buffered saline)
5. Cell counter and Trypan blue
6. 96-well plates
7. Pipettes, 1.5 ml tubes
8. TPCK-Trypsin (for Sendai virus infection, see SeV infection protocol)

Seed cells to 96-well plate

1

1. Check LLCMK2 cells to ensure they are in good condition, approximately 90% confluent.
2. Wash cells twice with PBS.
3. Add 3 mL 0.05% trypsin-EDTA to the T75 flask, incubate at room temparture for 3-4 minutes.
4. Add 7 mL TCM to the flask to stop trypsinization and collect cells.
5. Mix 10 µL cells and 10 µL Trypan blue, then add 10 µL mixture to a cell counter slide and count the cells.
6. Plate 20,000 cells in 100 µL of TCM media to each well. 3 rows of a 96-well plate are need for one test.
7. Incubate plates at 37 °C overnight.

Notes:

- Usually, LLCMK2 cells are used for SeV titration since most subsequent experiments are done with LLCMK2. However, if you plan to infect A549 cells or other cell lines with the virus stocks, it is recommended to do the titration on the same cell line you intend to use.
- The seeding number for LLCMK2 and A549 cells is similar, usually around 20,000 cells per well. This results in 95–100% confluence overnight, though this can vary depending on the condition of the cells. In my experiments, seeding numbers between 12,000 and 20,000 cells per well have been successfully used.

Titration

2

1. Prepare infection media: Add TPCK-trypsin to the infection media at a final concentration of 2 µg/mL. Prepare 30 mL of the media for each plate, ensuring sufficient volume for dilutions and replacing the TCM from the plate. (This step is for LLCMK2 cells, if you are using A549 cells, TPCK-trypsin is not needed).
2. Use the prepared media to do a 10-fold serial dilution from $1:10^1$ to $1:10^8$: Prepare 8 tubes. Add 450 µL of prepared media to each tube. Add 50 µL of virus to the first tube, vortex to mix thoroughly, then transfer 50 µL to the second tube. Repeat this process sequentially until the 8th tube.
3. Check the cells under a microscope to confirm they are healthy and evenly distributed.
4. Remove the TCM from the plate using a multichannel pipette.
5. Wash the cells by adding 200 µL of PBS to each well, then discard the PBS.
6. Add 100 µL of the prepared infection media (containing TPCK-trypsin) to each well.

7. Add 100 µL of the diluted virus to the cells, with each dilution to three wells (e.g., columns A-C as below).
8. Incubate the plate at 37 °C for 3–5 days.

	A	B	C	D	E	F	G	H	I	J	K	L
	Stock1 1:10^1	Stock1 1:10^1	Stock1 1:10^1	Stock2 1:10^1	Stock2 1:10^1	Stock2 1:10^1	...					
	Stock1 1:10^2	Stock1 1:10^2	Stock1 1:10^2	Stock2 1:10^2	Stock2 1:10^2	Stock2 1:10^2						
	Stock1 1:10^3	Stock1 1:10^3	Stock1 1:10^3	Stock2 1:10^3	Stock2 1:10^3	Stock2 1:10^3						
	Stock1 1:10^4	Stock1 1:10^4	Stock1 1:10^4	Stock2 1:10^4	Stock2 1:10^4	Stock2 1:10^4						
	Stock1 1:10^5	Stock1 1:10^5	Stock1 1:10^5	Stock2 1:10^5	Stock2 1:10^5	Stock2 1:10^5						
	Stock1 1:10^6	Stock1 1:10^6	Stock1 1:10^6	Stock2 1:10^6	Stock2 1:10^6	Stock2 1:10^6						
	Stock1 1:10^7	Stock1 1:10^7	Stock1 1:10^7	Stock2 1:10^7	Stock2 1:10^7	Stock2 1:10^7						
	Stock1 1:10^8	Stock1 1:10^8	Stock1 1:10^8	Stock2 1:10^8	Stock2 1:10^8	Stock2 1:10^8						

Read titration results

3 Read the results at 4dpi as below:

	A	B	C
	Positive wells of last row with signal	Dilution of last row with signal	TCID50 / 100uL
	+ + +	10-X	10^X + 0.7
	+ + -	10-X	10^X + 0.4
	+ - -	10-X	10^X - 0.1

X = row in which last signal occurs
+ = Fluorescent signal positive

Notes:

- The $TCID_{50}$ result is reported per 100 µL. To convert it to per mL, plus 1 to the X. For example, a $TCID_{50}$ of $10^{7.7}$/100 µL is equivalent to $10^{8.7}$ $TCID_{50}$/mL.
- For rSeV-C-eGFP, the $TCID_{50}$ results remain consistent from 3 dpi to 5 dpi. So, the results can be read on any of these days.