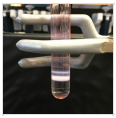

Reovirus Viral Purification

Version 2

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ABSTRACT

Purification of mammalian orthoreovirus by CsCl gradient

EXTERNAL LINK

https://doi.org/10.1371/journal.ppat.1006768

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Berger AK, Yi H, Kearns DB, Mainou BA (2017) Bacteria and bacterial envelope components enhance mammalian reovirus thermostability. PLoS Pathog 13(12): e1006768. doi: 10.1371/journal.ppat.1006768

Reovirus Purification BAM lab.docx

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

BEFORE STARTING

Reagents

HO Buffer

1 mL 1 M Tris, p 7.4
5 mL 5 M NaCl
67 uL B-ME
Water to 100 mL
Filter sterilize through 0.2 micron membrane

Dialysis Buffer

120 mL 5M NaCl
60 mL 1M MgCl2
40 mL 1M Tris, pH 7.4
water to 4 L
Filter sterilize through 0.2 micron membrane

1.2 g/cm^3 CsCl

33.3 g CsCl
Dialysis buffer to 100 mL
Filter sterilize through 0.2 micron membrane

1.4 g/cm^3 CsCl

67 g CsCl
Dialysis buffer to 100 mL
Filter sterilize through 0.2 micron membrane

344059 Tube, Thinwall, Ultra-Clear™, 13.2 mL, 14 x 89 mm
86703 DIALYSISTUBING SP1 8K 10MM 15M
880111 S/P CLOSURES 35MM GREEN 10/PK
21009-284 TUBE CENT AUTOCLAV 50ML PK10 3117-0500

1 Pellet 4x10^8 spinner-adapted L929 at 2000 x g for 10 min at 4°C.

2 Remove supernatant (can be added back to 1 L bottle to be used during infection).

3 Resuspend cells in total volume of 40 ml (virus in Joklik's Minimum Essential Media without supplements, JMEM).
a. Adsorb for 1 h at room temperature with passage 2 or viral prep supernatant at an MOI of 10 PFU/cell with gentle shaking on orbital shaker.

4 Add adsorption mixture to 760 ml JMEM supplemented with 5% FBS, 2mM L-Glutamine, 100 U penicillin per ml, 100 ug streptomycin per ml, and 0.25 mg per ml amphotericin B

5 Incubate on a spinner plate at 34-37°C with environmental CO_2 for 72 h.

6 Spin at 2500 x g for 10 min at 4°C.

7 Remove supernatant and resuspend cells in 7 mL of HO buffer. Suspension may be stored at -20-80°C at this step. If using immediately one freeze/thaw cycle is recommended. Supernatants of infections started with passage 2 reovirus can be stored at 80°C and used for future viral purifications.

8 Thaw HO suspension on ice.

9 Add 100 ul 10% DOC per tube and incubate on ice for >30 min, vortexing every 10 min.

10 Add 2.5 mL Vertrel XF.

11 Sonicate on ice for 1 min to disrupt cells and place on ice.

12 Add additional 2.5 mL Vertrel XF.

13 Sonicate on ice for 1 min to disrupt cells and place on ice.

14 Centrifuge at 9700 x g for 10 min at 4°C.

15 Transfer aqueous (top) layer to a clean tube and discard pellets.

16 Add 2.5 mL Vertrel XF.

17 Sonicate on ice for 1 min to disrupt cells and place on ice.

18 Centrifuge at 9700 x g for 10 min at 4°C.

19 During second centrifugation step prepare CsCl gradient:
a. Add 2.5 mL 1.2 g/mL CsCl and gently underlay with 2.5 mL 1.4 g/mL CsCl being careful to not mix layers.

20 Carefully layer aqueous (top) fraction onto CsCl gradient. Balance tubes with HO buffer.

21 Spin at 25000 RPM overnight at 5°C.

22 Wipe bottom of tube with ethanol.

23 Puncture the bottom of the tube with an 18.5-gauge needle.

24 Collect virus fraction (bottom band) and top-component (top band) into a clean tube.

25 Dialyze exhaustively against 400-500 mL cold dialysis buffer for at least 24 h at 4°C. (Change buffer after 1 h, 4 h, and next morning).

26 Transfer to new tube.

27 Determine particle density (1 OD260 = 2.1 x 1012 particles/mL = 185 ug viral protein/mL).

28 Store at 4°C.