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Protocol status: Working

Reovirus Viral Purification V.1

PLOS Pathogens

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ABSTRACT

Purification of mammalian orthoreovirus by CsCl gradient

ATTACHMENTS

[Reovirus Purification
BAM lab.docx](#)

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BEFORE START INSTRUCTIONS

Last Modified: Feb 21, 2018

Reagents

PROTOCOL integer ID:
8688

Keywords: Reovirus, virus,
purification, CsCl

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

Dialysis Buffer

120 mL 5M NaCl
60 mL 1M MgCl₂
40 mL 1M Tris, pH 7.4
water to 4 L

1.2 g/cm³ CsCl

33.3 g CsCl
Dialysis buffer to 100 mL
Filter Sterilize through CN membrane

1.4 g/cm³ CsCl

67 g CsCl
Dialysis buffer to 100 mL
Filter Sterilize through CN 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 4x10⁸ 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 CO₂ 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 ($1 \text{ OD}_{260} = 2.1 \times 10^{12} \text{ particles/mL} = 185 \text{ ug viral protein/mL}$).

Store at 4°C.