Reovirus Viral Purification

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Abstract

Purification of mammalian orthoreovirus by CsCl gradient

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Before start

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

Dialysis Buffer

120 mL 5M NaCl

60 mL 1M MgCl2

40 mL 1M Tris, pH 7.4

water to 4 L

1.2 g/cm^3 CsCl

33.3 g CsCl

Dialysis buffer to 100 mL

Filter Sterilize through CN membrane

1.4 g/cm^3 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

Protocol

Step 1.

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

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

Step 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.

Step 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

Step 5.

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

Step 6.
Spin at 2500 x g for 10 min at 4°C.

Step 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.

Step 8.
Thaw HO suspension on ice.

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

Step 10.
Add 2.5 mL Vertrel XF.

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

Step 12.
Add additional 2.5 mL Vertrel XF.

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

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

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

Step 16.
Add 2.5 mL Vertrel XF.

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

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

Step 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.

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

Step 21.
Spin at 25000 RPM overnight at 5°C.

Step 22.
Wipe bottom of tube with ethanol.

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

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

Step 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).

Step 26.
Transfer to new tube.

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

Step 28.
Store at 4°C.