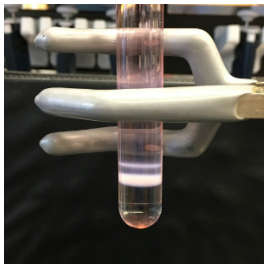
Nov 14, 2018 Version 3

Reovirus Viral Purification V.3



PLOS Pathogens

DOI
dx.doi.org/10.17504/protocols.io.vj7e4rn

Bernardo Mainou[1]

[1]Emory University

Bernardo Mainou
Emory University

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.vj7e4rn

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

Protocol Citation: Bernardo Mainou 2018. Reovirus Viral Purification. **protocols.io https://dx.doi.org/10.17504/protocols.io.vj7e4rn**

Manuscript citation:
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

Protocol status: Working
We use this protocol and it's working

Created: November 14, 2018

Last Modified: November 14, 2018

Protocol Integer ID: 17759

Keywords: Reovirus, virus, purification, CsCl, reovirus viral purification purification of mammalian orthoreovirus, reovirus viral purification purification, mammalian orthoreovirus

Abstract

Purification of mammalian orthoreovirus by CsCl gradient

Attachments

DOCX

Reovirus Purificatio...

131KB

Troubleshooting

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 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 × 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 4×10^8 spinner-adapted L929 at 2000 x g for 00:10:00 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 01:00:00 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 °C - 37 °C with environmental CO_2 for 72:00:00 .

6 Spin at 2500 x g for 00:10:00 at 4 °C .

7 Remove supernatant and resuspend cells in 7 mL HO buffer . Suspension may be stored at -20 °C - 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 µL 10% DOC per tube and incubate on ice for > 00:30:00 , vortexing every 00:10:00 .

10 Add 2.5 mL Vertrel XF .

11 Sonicate on ice for 00:01:00 to disrupt cells and place on ice.

12 Add additional 2.5 mL Vertrel XF .

13 Sonicate on ice for 00:01:00 to disrupt cells and place on ice.

14 Centrifuge at 9700 x g for 00:10:00 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 00:01:00 to disrupt cells and place on ice.

18 Centrifuge at 9700 x g for 00:10:00 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 mL - 500 mL cold dialysis buffer for at least 24:00:00 at 4 °C. (Change buffer after 01:00:00, 04:00:00, and next morning).

26 Transfer to new tube.

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

28 Store at 4 °C Storage.