Cesium Chloride Dialysis for Viruses

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Abstract

Version 3

This protocol describes the use of Cesium Chloride to purify viruses with an optional dialysis step to remove the CsCl. DNase I treatment, CsCl purification, and sucrose purification methods were compared using replicated viral metagenomics in Hurwitz et al. 2012.

References:
Hurwitz, B.L., Deng, L., Poulos, B.T., & Sullivan, M.B. (2012). Evaluation of methods to concentrate and purify ocean virus communities through comparative, replicated metagenomics. Environ Microbiol. 15(5), 1428–1440. doi:10.1111/j.1462-2920.2012.02836.x.

Citation: Matthew Sullivan Cesium Chloride Dialysis for Viruses. **protocols.io**
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Guidelines

This protocol is part of a larger collection of Cesium-Chloride related protocols. This is number (4) of (4):
1. Cesium Chloride Gradients
2. CsCl Step Gradient to Purify Phage
3. Cesium Chloride and DNA Extraction of Viruses using Wizard Prep Columns
4. Cesium Chloride Dialysis for Viruses

To prepare a CsCl solution of a particular density, the percent by weight of CsCl can be calculated by the formula:
% wt/wt = 137.48 - 138.11/p
where 'p' is the desired density. For example, for p = 1.7 g/mL, use 56.24g CsCl and 43.76 mL H2O.

Needed:

- Milli-Q Water
- MTN 100 (NaCl, Tris-Cl (pH 7.5), MgCl2)
- Slide-A-Lyzer dialysis casettes
- Tris Base

If it is necessary to remove the CsCl, the virus preparation should be dialyzed against a buffer containing sodium chloride and magnesium chloride. The virus should be dialyzed against 4 changes of buffer with the first at a high salt concentration, then at an intermediate salt concentration and finally twice using the final salt concentration. Dialysis should be at 300x the sample volume for 30 min at room temperature. The dialysis membrane should have a 10,000 molecular weight cut-off (10K

MWCO).

Protocol

Prepare Dialysis Buffers using Milliq or Other Purified Waters

Step 1.
Prepare MTN100 with 5x NaCl

PROTOCOL

. MTN100 with 5x NaCl

CONTACT: VERVE Team

Step 1.1.
Mix 3M (175.32g) NaCl with 0.1M (12.7g) Tris-Cl, pH 7.5

Step 1.2.
Add this mixture to 0.1M (20.33g) $MgCl_2$-$6H_2O$

Step 1.3.
Add 2.36g of Tris Base

Step 1.4.
Bring volume to 1 L with MilliQ water

Prepare Dialysis Buffers using Milliq or Other Purified Waters

Step 2.
Prepare MTN100 with 3x NaCl

PROTOCOL

. MTN100 with 3x NaCl

CONTACT: VERVE Team

Step 2.1.
Mix 1.8M (105.2g) NaCl with 0.1M (12.7g) Tris-Cl, pH 7.5

Step 2.2.
Add this mixture to 0.1M (20.33g) $MgCl_2$-$6H_2O$

Step 2.3.
Add 2.36g of Tris Base

Step 2.4.
Bring volume to 1 L with MilliQ water

Prepare Dialysis Buffers using Milliq or Other Purified Waters

Step 3.
Prepare MTN100 with 1x NaCl

PROTOCOL

. MTN100 with 1x NaCl

CONTACT: VERVE Team

Step 3.1.
Mix 0.6M (35.07g) NaCl with 0.1M (12.7g) Tris-Cl, pH 7.5

Step 3.2.
Add this mixture to 0.1M (20.33g) $MgCl_2$-$6H_2O$

Step 3.3.
Add 2.36g of Tris Base

Step 3.4.
Bring volume to 1 L with MilliQ water

Prepare Dialysis Buffers using Milliq or Other Purified Waters

Step 4.
Bring volume to 1L with Milli-Q water.

Loading and Dialysis of Cassettes

Step 5.
Use Slide-A-Lyzer dialysis casettes (Pierce #66425 or #87730; 10K MWCO, 0.5-3.0ml) and follow the manufacturer's directions for loading and dialysis of cassettes.

Step Dialyze

Step 6.
Step dialyze for 30 min with the 5x NaCl MTN buffer.

DURATION
00:30:00

Step Dialyze

Step 7.
Step dialyze for 30 min with the 3x NaCl MTN buffer.

DURATION
00:30:00

Step Dialyze

Step 8.
Step dialyze for 30 min with the two changes of the 1x NaCl MTN, using at least 300 volumes of buffer to sample.

DURATION
00:30:00

Step Dialyze

Step 9.
Stir gently at room temperature

NOTES
VERVE Team 14 Jul 2015
Keep dark, if possible.

Post Dialysis

Step 10.
Remove the sample according to manufacturer's directions

Post Dialysis

Step 11.
Transfer to sterile, low bind tubes.

Post Dialysis

Step 12.

Store dialyzed particles at 4°C.