Improved Method For Adeno and Lenti- Virus Purification and Titration

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Abstract

Highly purified virus is a requirement for many downstream applications as cellular debris and proteins derived from culture media in crude virus preparation can be toxic to target cells or can cause immunogenic reactions when used for in vivo injection. Conventional virus purification methods based on density gradient ultracentrifugation (most widely used sucrose or cesium) require technical expertise and are time-consuming. These methods often result in low virus recovery.

We report here a fast and easy method for Adenovirus and Lentivirus purification. In our purification method, viral particles are captured on chemically modified membranes. Our specific wash and elution protocol allows for a quick and efficient purification resulting in high yields of pure virus and doesn't affect infectivity.

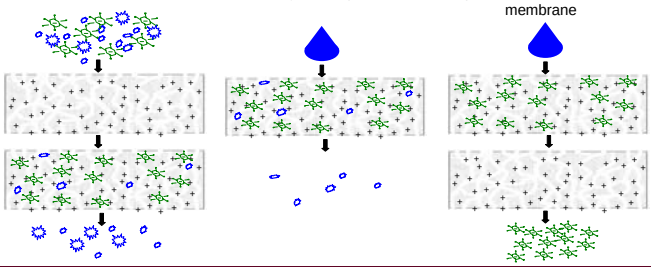
In addition, we developed an accelerated method for virus titration and detection. Standard plaque assay for determining infectious titers is tedious, and takes one or weeks. Our method is ELISA based, can be completed within two hours, and produces consistent results. The method can be used to characterize crude virus preparations from cell lysates as well as purified virus.

Virus Purification: Workflow

STEP 1: bind
Add crude virus to purification filter membrane

STEP 2: wash
Add wash buffer to rinse off weakly bound proteins

STEP 3: elute
Add elution buffer to remove purified virus from filter membrane



Adenovirus Purification: Results

Adenovirus used for purification: Ad5.CMV5-GFP (Qbiogene)

Fig 1a

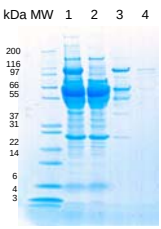


Fig 1b

Fig. 1a: *SDS PAGE, Coomassie staining*:
Lane 1= Clarified Adenovirus sample, Lane 2 = Unbound Material, Lane 3 = wash, Lane 4 = Eluate from Millipore virus purification device

Fig. 1b: *Western Blot with anti-hexon antibodies, MAB8044(Millipore)*:
Lane 1 = Starting Material, Lane 2 = Unbound material, Lane 3 = wash, Lane 4 = Eluate from Millipore virus purification device

Fig 1c

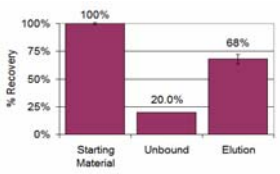


Fig 1c: *% recovery using Millipore virus purification device:*
Input was 3.44 E+10 IVP (as determined by GFP expression) and2.33 E+10 IVP or 68 % was recovered after purification.

Lentivirus Purification: Results

Lentivirus used for purification: GFP-VTK945,VSV-G pseudotype (provided by UNC Vector Core and Dr. R. Jude Samulski)

Fig. 2a

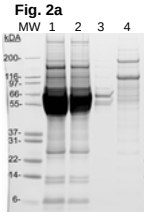


Fig 2b

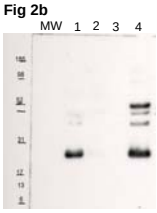


Fig. 2a: *SDS PAGE, Spyro Ruby staining*:
Lane 1= Clarified Lentivirus sample, Lane 2 = Unbound Material, Lane 3 = Wash, Lane 4 = Eluate from Millipore virus purification device

Fig. 2b: *Western Blot with anti HIV-p24 antibodies, MAB 8790 (Millipore)*:
Lane 1 = Starting Material, Lane 2 = Unbound material, Lane 3 = Wash, Lane 4 = Eluate from Millipore virus purification device

Fig 2c

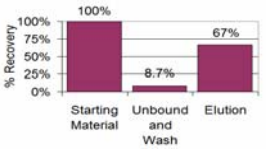


Fig 2c: *% recovery using Millipore virus purification device:*
Input was 7.2 E+07 IVP (as determined by GFP expression) and 4.9 E+07 IVP or 67 % was recovered after purification.

Fig 2d

	Unpurified Lentivirus	Purified Lentivirus
VP/ml	3.56E+10	2.254E+11
IVP/ml	2.81E+06	1.29E+07
ratio VP/IVP	1.27E+04	1.75E+04

Fig 2d: *ratio VP/IVP before and after purification:*
IVP/ml of unpurified and purified Lentivirus was determined by GFP expression. VP/ml was determined by HIV p24 ELISA (QuickTiter™ Lentivirus Quantitation Kit, Cell Biolabs, Inc., cat# VPK-108-HIC p24)

ELISA on MultiScreen$_{HTS}$® Plate: Workflow

Step 1: Add antigen (10 min)

Step 2: Filter & Wash 3X (3 min)

Step 3: Block (10 min)

Step 4: Add Primary Ab (10 min)

Step 5: Filter & Wash 3X (3 min)

Step 6: Add Secondary Ab-AP (10 min)

Step 7: Filter & Wash 3X (3 min)

Step 8: Add AP Chemiluminescent Substrate (10 min)

MultiScreen$_{HTS}$ IP plate (cat# MSIP S4W 10, Millipore), MultiScreen$_{HTS}$ Vacuum Manifold® (cat# MSVM HTS 00, Millipore), Millipore Immobilon® Western AP Chemiluminescent Substrate (cat# WBKD S01 00, Millipore)

MultiScreen$_{HTS}$ plate ELISA Results: Adenovirus Titration Curve

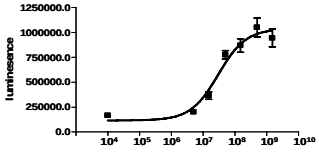
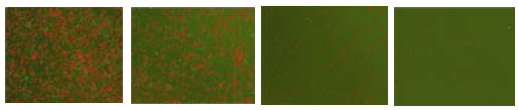


Figure 3: *MultiScreen plate ELISA with an anti-hexon Ab (cat# MAB805, Millipore)* using different concentration of adenovirus was performed at day 3 post infection. Data were fitted into a sigmoidal curve using Prism software (GraphPad Softhware). MultiScreen plate ELISA resulted in a titration curve comparable to GFP results (see Figure 4).

Image Analysis for GFP-Adenovirus Titration Area Selection Using Image-Pro ® Plus software



10^7 IVP/ml 10^6 IVP/ml 10^5 IVP/ml 10^4 IVP/ml

Figure 4a: *(40X Magnification) Analysis of GFP expression 3 days post infection*:
96-well plates were seeded with 10,000 HEK293A cells per well in 2% FBS containing media. Cells were infected with various concentrations of adenovirus expressing GFP (Ad5.CMV5-GFP, Qbiogene). Pictures were taken on day 3 after infection. Images show area selection using Image-Pro Plus software, MediaCybernetics.

Titration on 3rd, 4th, and 5th Day Post-Infection

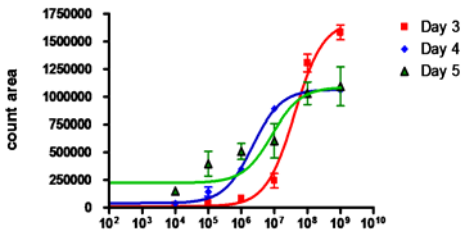


Figure 4b: *Data from Image-Pro software was fitted into a sigmoidal curve using Prism ™ software (GraphPad software):* Based on the resulting sigmoidal curves obtained at 3 different time points, day 3 was identified as an optimal time point for Adeno-GFP titration.

Conclusions

- **Purification of adeno- and lentivirus by membrane chromatography results in highly pure and high yield virus and doesn't affect infectivity**
- **Quantification by GFP-Adenovirus Image Analysis or Adenovirus ELISA can be performed at day 3 post-infection in comparison to TCID$_{50}$ which requires a 10 days infection period**
- **MultiScreen$_{HTS}$ titration method is a fast alternative to traditional ELISA and can be performed in about 2 hours**