New Workflow for Lentivirus Purification, Concentration, and Immunodetection

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Abstract

Lentivirus is a negatively charged, enveloped, single stranded RNA virus from the *Retroviridae* family that is often used as a vector to transport genetic material into cells. These viral vectors can be used for genetic modification, RNAi, gene therapy, and vaccine production. Before viral preparations/propagations can be used for any of these applications, researchers need to purify their virus sample.

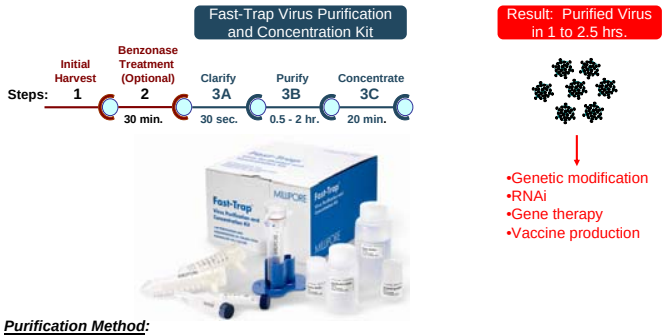
Traditionally, time-consuming density gradient centrifugation separation and/or chromatographic techniques have been used. The lab-scale chromatographic devices are commonly syringe or column-based. To process the virus sample, these devices require hand pressure or gravity; this may lead to messy and to potentially unsafe handling conditions during assembly and disassembly.

A new workflow has been developed to clarify, purify, and concentrate/buffer exchange a crude lentivirus sample. For added safety and improved handling, the clarification and purification steps are performed in a closed vacuum-based device. This purification produces high recovery of virus particles in about one hour with similar or improved results as compared to traditional methods. Purity was visualized by gel electrophoresis and confirmed by western blotting using an innovative vacuum-based immunodetection system that allows detection of the protein of interest in less than forty minutes.

Here we show the results of the purification and the immunodetection of a Lentivirus-VSVG pseudotype that encodes green fluorescent protein (GFP). We demonstrate the viral titer, the percent recovery of infectious particles, and the purity of the virus sample.

Lentivirus Fast-Trap™ Virus Purification & Concentration Kit combined with SNAP i.d.™ Immunodetection enables results in less than three hours.

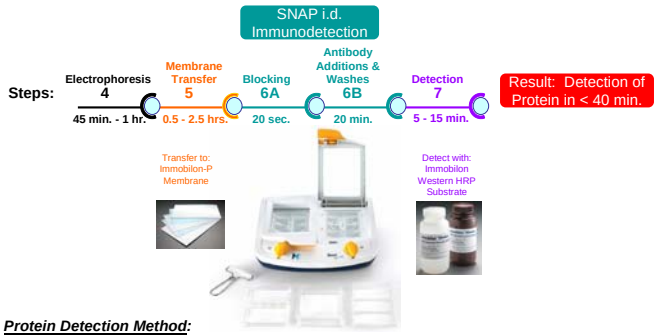
New Workflow: Virus Purification & Concentration



Purification Method:

- **Step 1**: Initial harvest of lentivirus
- **Step 2**: (Optional) Benzonase treatment 30 minutes at 37 ˚C
- **Step 3**: **Purification with Fast-Trap Lentivirus Purification & Concentration Kit (Millipore #FTLV00003):**
  - **Step 3A**: Clarify using provided 0.45 µm HV Steriflip® filter unit by vacuum filtration
  - **Step 3B**: Purify virus using Fast-Trap purification device & buffers
  - **Step 3C**: Concentrate/buffer exchange of eluted lentivirus with supplied 100 kDa Amicon® Ultra device.

Immunodetection Workflow With the SNAP i.d. Protein Detection System



Protein Detection Method:

- **Step 4**: Electrophoresis-Samples denatured & reduced before SDS-PAGE
- **Step 5**: Transfer to Immobilon®-P membrane (Millipore #IPVH07850)
- **Step 6**: **Immunodetection with SNAP i.d. Protein Detection System (Millipore #WBAVDBASE)**
  - **Step 6A**: Apply blocking reagent containing Tween® 20 surfactant and vacuum filter
  - **Step 6B**: Apply antibodies (primary, secondary) and wash between antibody additions
- **Step 7**: Add Immobilon® Western Chemiluminescent HRP substrate (Millipore #WBKLS0500) for chemiluminescence detection of protein of interest and visualize immunoreactive proteins

Performance of the Fast-Trap Lentivirus Purification & Concentration Kit and SNAP i.d. Protein Detection Workflow

Methods

Fast-Trap Purification

Virus: Crude lentivirus (University of North Carolina [UNC] Vector Core) was benzonase treated, clarified, purified, and concentrated using the new Fast-Trap kit workflow

SDS-PAGE

Samples: Virus samples normalized for protein concentration, denatured and reduced
Gel: NuPAGE® 4 – 12% Bis-Tris gel (Invitrogen) 200 V, 35 minutes
Gel Markers: M = Mark12™ unstained standard or MagicMark™ XP Western standard (Invitrogen)
Stain: SYPRO® Ruby stain (Invitrogen) overnight, destain, and visualize, or membrane transfer for immunodetection

Membrane Transfer

Transfer: Semi-dry transfer method (BioRad Trans-Blot® SD Semi-dry Transfer cell) to Immobilon-P membrane at 10 V, 35 minutes with Tris-Glycine buffer

SNAP i.d. Immunodetection

Blocking: Add blocking buffer (0.5% non-fat dry milk in Tris Buffered Saline with 0.1% Tween-20 surfactant, TBST) and vacuum
1° Antibody: Probe with Mouse anti-HIV, p24 (Millipore #MAB8790) diluted 1:13,000 in blocking buffer, and incubate for 10 minutes
Wash: Wash with TBST buffer using constant vacuum
2° Antibody: Add Goat anti-Mouse IgG, HRP-conjugate (Millipore #AP124P) diluted 1:10,000 in blocking buffer, and incubate for 10 minutes
Wash: Wash with TBST buffer using constant vacuum
Detect: Incubate 5 minutes with Immobilon Western Chemiluminescent HRP substrate, and visualize by exposing to x-ray film

Recovery of Infectious Particles with the Fast-Trap Purification and Concentration Kit

Figure 1

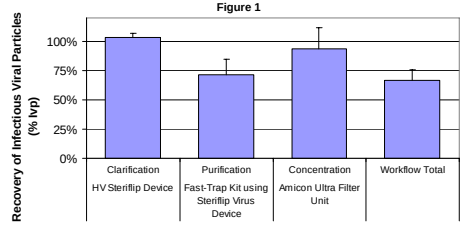


Figure 1: Human Embryonic Kidney (HEK 293A, Invitrogen) cells were transduced with serial dilutions of crude, clarified, purified, and concentrated viral fractions. After three days, fluorescence of virus-transduced cells expressing GFP were quantified by image analysis and/or manual counting. Viral titer and percent recovery of infectious particles (lvp) were determined. All of the lentivirus was recovered during clarification step (input ~ 1 x 10^8 lvp). Clarified lentivirus (~ 3.9 x 10^7 lvp) was purified using the Fast-Trap purification device, and efficient recovery observed (avg. 72%). The eluted virus (~ 2 x 10^7 lvp) was concentrated/buffer exchanged and high recovery achieved (~2 x 10^7, avg. 94%). The total lentivirus recovery for this workflow was ~ 67%.

Sample Purity & Capsid Protein P24 Detection

Figure 2

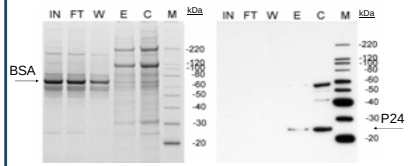


Figure 2: SYPRO Ruby Stain of SDS-PAGE. Using equal protein load (3 µg per lane), the presence of serum protein bovine serum albumin (BSA) was observed in the input (IN), flow through (FT) and wash (W) fractions. A reduction of BSA was observed in both the eluted (E) and concentrated (C) fractions.

Figure 3

Figure 3: Detection of Capsid Protein P24 by SNAP i.d. Immunodetection. Using equal protein load (3 µg per lane), the presence of lentiviral Capsid Protein P24 was confirmed in the eluted (E) and concentrated (C) fractions.

Ratio of Infectious to Non-Infectious Viral Particles

Table 1

Average	Unpurified Lentivirus	Purified Lentivirus
Concentration by ELISA (vp/mL x 10^{10})	3.6	22.5
Infective particle Input (lvp x 10^6)	2.8	12.9
Ratio (vp/lvp x 10^4)	1.3	1.7

Table 1: The ratio for unpurified and purified lentivirus (using the Fast-Trap Purification and Concentration Kit) was determined by Enzyme-Linked ImmunoSorbent Assay (ELISA) Quick Titer™ Lentivirus Quantitation kit (Cell Biolabs). This ratio is maintained when using Fast-Trap Lentivirus Purification and Concentration kit.

Lentivirus Purification: Comparison of the Fast-Trap Kit to Other Formats/Methods (Syringe, Column, Precipitation, & Traditional Sucrose Gradient)

Processing Time & Virus Recovery

Table 2

Purification Format	Key	Processing Volume (ml)	Processing Time (min.)	Recovery of Infectious Viral Particles, lvp (%)
Vacuum, closed system	Fast: Fast-Trap Lentivirus	9	6	57%
Gravity syringe	S1	9	11	9%
Gravity syringe	S2	9	10	21%
Gravity syringe	S3	9	11	29%
Column	Col	9	300	38%
Precipitation/Centrifugation	Ppt	10	12 Hr. + 35	57%

Table 2: Crude lentivirus (Input 1.4 x 10^7 lvp) was purified with the Fast-Trap Virus Purification and Concentration Kit, three types of gravity syringe chromatographic columns (S1, S2 & S3), one column (Col), and one precipitation (Ppt) method (following manufacturer's instructions). The table compares overall performance and processing time (for bind/wash/elute, or precipitation/centrifugation steps only). The Fast-Trap purification method has the highest recovery and the shortest processing time. The closed, vacuum-based Fast-Trap's purification device was easy to handle without the messy assembly/disassembly process or flow regulation that some formats require.

Sample Purity & Capsid Protein P24 Detection

Figure 4

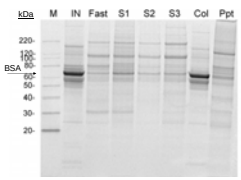


Figure 4: SYPRO Ruby Stain of SDS-PAGE gel. Purity of input (IN) and elution fractions were visualized on the SDS-PAGE gel loading equal protein (5 µg per lane). The gel demonstrates reduction of BSA in the Fast-Trap Virus method (Fast) and other formats. These include three syringe-based (S1, S2, & S3), one column (C), and one precipitation (Ppt). Contaminating protein (BSA) is observed in the column (Col) and precipitation (Ppt) methods.

Figure 5

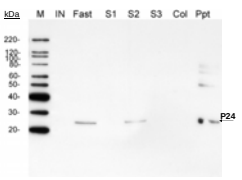


Figure 5: Detection of Capsid Protein P24 by SNAP i.d. Immunodetection. Using 5 µg of protein per lane, the presence of the lentivirus Capsid Protein P24 was confirmed in the Fast-Trap Virus (Fast), Syringe S2, and precipitation (Ppt) methods.

Fast-Trap Virus Purification and Concentration Kit versus Traditional Sucrose Gradient Method

Figure 6

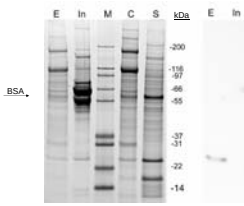


Figure 6: SYPRO Ruby Stain of SDS-PAGE gel. Using equal protein load (3 µg per lane), purity of the input (In), the Fast-Trap kit's eluted (E) and concentrated (C) fractions are compared to a traditional sucrose (S) gradient method. Removal of BSA is observed in the purified samples.

Figure 7

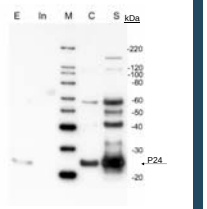


Figure 7: Detection of Capsid Protein P24 by SNAP i.d. Immunodetection. Using equal protein load (3 µg per lane), the presence of lentivirus Capsid Protein P24 was confirmed in the eluted (E), concentrated (C), and sucrose gradient method (S, provided by UNC Vector Core).

Conclusions

- Results can be generated in less than three hours using the Fast-Trap Virus Purification and Concentration kit followed by SNAP i.d. immunodetection
- Fast-Trap Virus Purification and Concentration Kit:
  - Enables efficient recovery of high titer lentivirus
  - Maintains ratio of non-infective to infective viral particles
  - Outperforms other chromatographic and precipitation formats for processing time, percent recovery of infectious particles, purity, and handling
  - Provides an alternative to precipitation, column or chromatography-based, and traditional gradient purification methods
- Vacuum-based SNAP i.d. Protein Detection System:
  - Shortens immunodetection process to ~ 30 minutes
  - Offers a faster alternative to traditional methods without consumption of additional reagents or loss of sensitivity

Acknowledgements

- UNC Vector Core & Dr. R. Jude Samulski for providing lentivirus samples