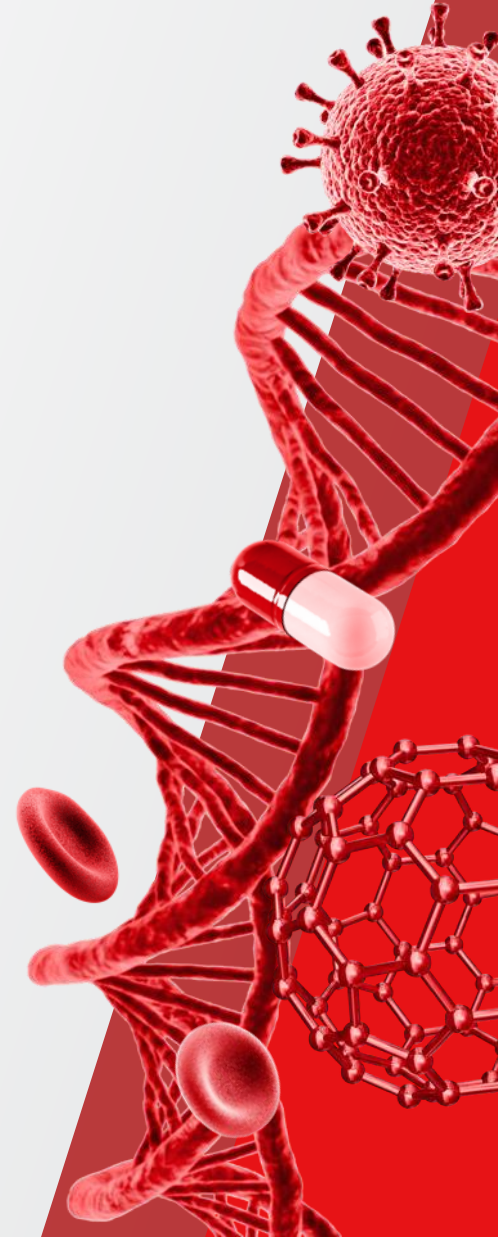


CaptureSelect™ Lenti VSVG Affinity Matrix (RUO)

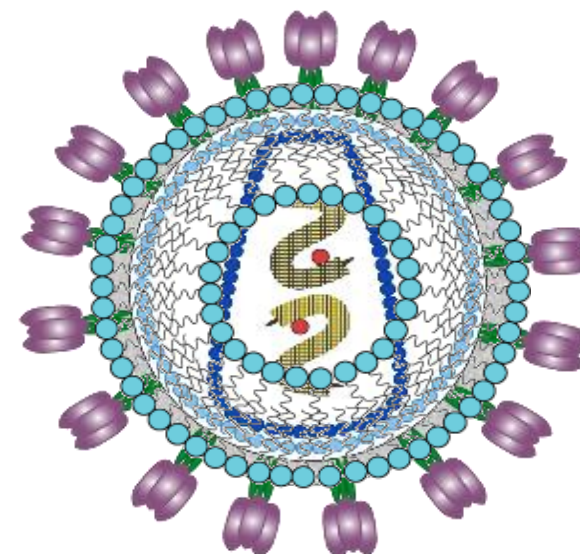
Product Launch July 2022 – Update Sept 2022

 The world leader in serving science



CaptureSelect Lenti VSVG affinity matrix

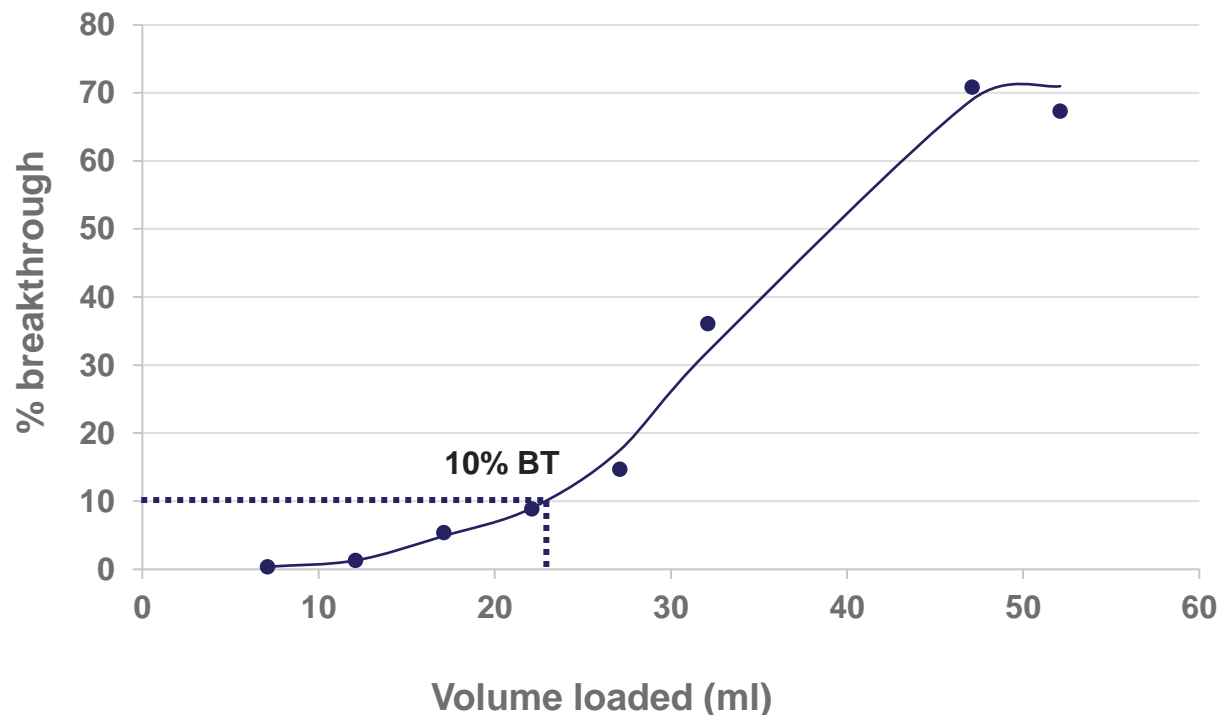
- Designed to bind VSV-G pseudotyped Lentiviral vector particles from suspension cultures
- High recovery and purity in a single step
- Gentle elution conditions, based on Arginine, to retain infectivity of the lentivirus particles
- A scalable affinity purification method based on an agarose base-bead
- Non-animal derived



**Designed to help increase productivity and efficiency
in the downstream process of lentiviral vectors**

Dynamic Binding Capacity – 1mL column

Dynamic Binding Capacity



DBC is determined by P24 total particle ELISA

Fraction	Volume (mL)	TP/mL	C/C0
Start		3.98E9	
1	7.1	1.55E7	0.39
2	12.1	5.24E7	1.32
3	17.1	2.14E8	5.39
4	22.1	3.53E8	8.88
5	27.1	5.85E8	14.7
6	32.1	1.44E9	36.09
7	47.1	2.82E9	70.84
8	52.1	2.68E9	67.33

24.6 mL

- 10% breakthrough ($C/C_0 = 10\%$) estimated from the curve at 24.6 ml loading
- This relates to $9.78E10$ total particles/ml resin (= $1E11$)

Dynamic Binding Capacity at 10% breakthrough is $1E11$ total particles/mL resin

Chromatography conditions

Endonuclease
treatment



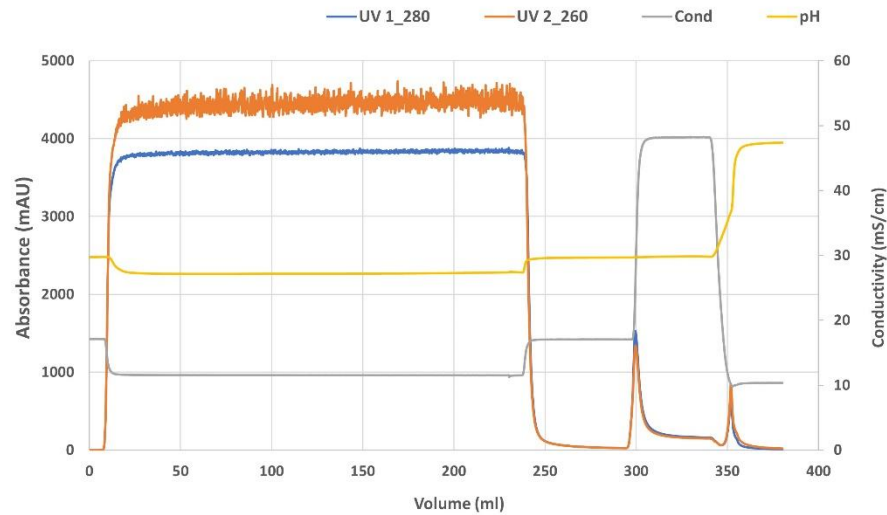
LV clarification
using microfiltration
(0.4 μm)



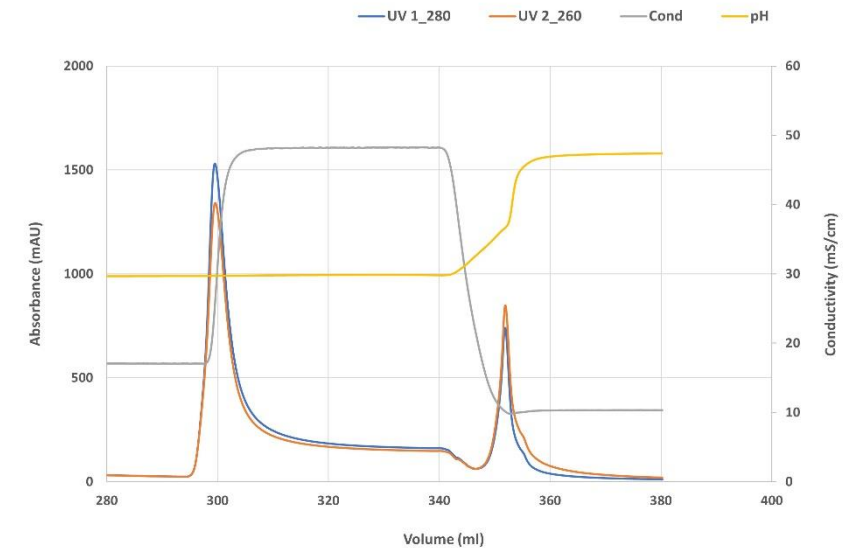
CaptureSelect
Lenti VSVG
affinity matrix

- Column 1.6x 5cm (10mL)
- Flow rate 150 cm/h
- Contact time 2 min

Binding/equilibration buffer: 50 mM HEPES, 150 mM NaCl pH 7.5
Elution buffer: 50 mM HEPES, 150 mM NaCl, 0.8 M Arginine pH 7.5
Strip buffer: 50 mM Sodium Phosphate pH 12



Chromatographic profile

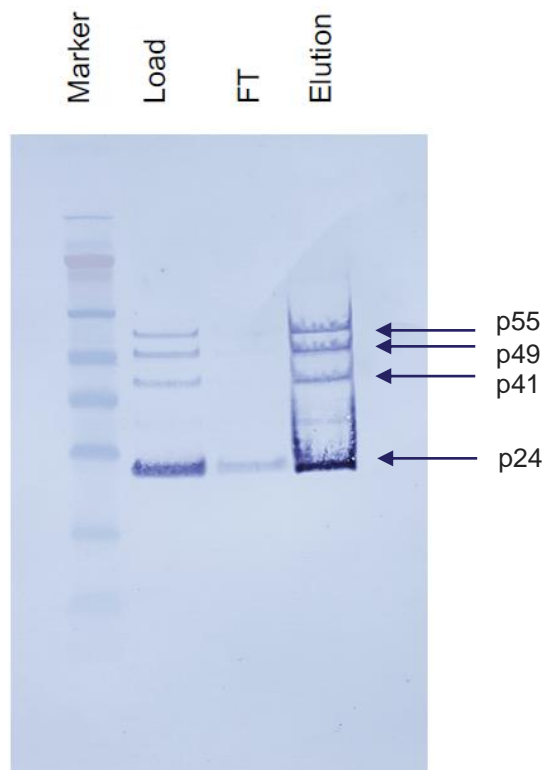


Elution close-up

The CaptureSelect Lenti VSVG affinity matrix demonstrates an efficient elution profile

Comparison of total particle to infectious particle ratios

P24 – WB pattern



Total particle (TP) and infectious particle (IP) ratio

Sample	TP/mL	IP/mL	TP/IP ratio
1. Feed	1.10E10	7.98E7	138
1. Flow through	3.25E8	8.30E5	392
1. Elution	4.44E10	4.42E8	100
2. Feed	1.11E10	9.00E7	165
2. Flow through	1.28E9	5.45E6	245
2. Elution	2.6E10	4.66E8	71

n=2

- The eluted fractions show a more than 5-fold increase of the infectious particle concentration compared to the load

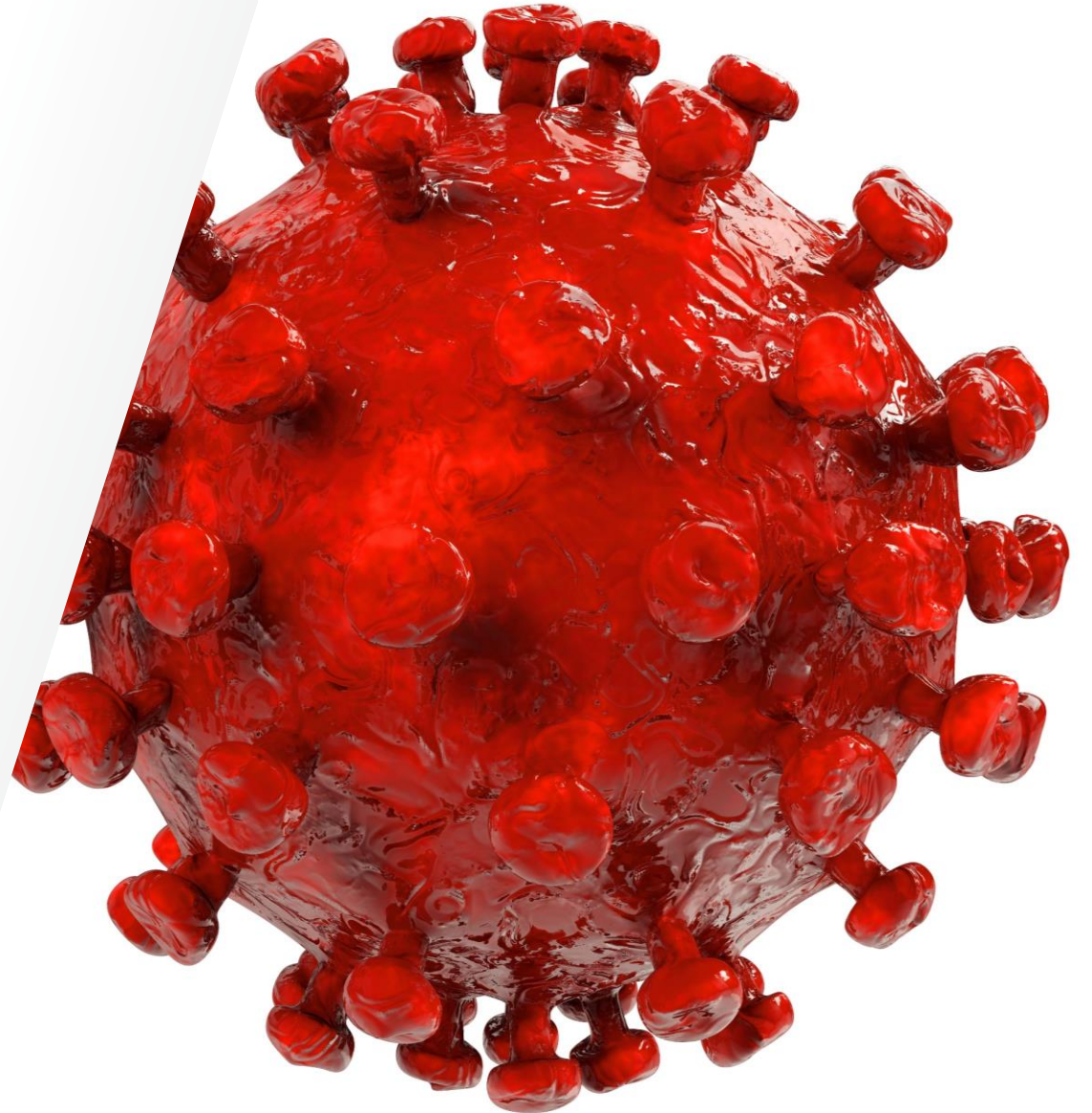
The concentration of infectious particles in the elution fraction has been enriched through purification using the Lenti-VSVG resin

Recovery of infectious particles (n=2)

Sample	Volume (mL)	IP/mL	TU (Transduction units)	Recovery	HCP removal	Total DNA removal
1. Feed	250	7.98E7	1.99E10			
1. Flow through	258	8.30E5	2.14E8			
1. Elution	22.5	4.42E8	9.95E9	49.9%	98.7%	80.2%
2. Feed	230	9.00E7	2.07E10			
2. Flow through	240	5.45E6	1.31E9			
2. Elution	25.6	4.66E8	1.19E10	57.7%	97.1%	96.5%

Recovery of infectious particles after purification using the Lenti VSGV resin is 50-60%

CaptureSelect Lenti VSVG resin characteristics



Lenti VSVG resin characteristics and available products

*For efficient purification of Lentivirus particles from suspension cultures,
pseudotyped with VSV-G*

MAIN RESIN CHARACTERISTICS

Matrix: agarose-based, epoxide activated

Average particle size: $65 \pm 10 \mu\text{m}$

Ligand: CaptureSelect Lenti VSVG affinity ligand

Ligand coupling method: epoxide

Binding capacity: $\sim 1\text{E}11$ total particles/ml matrix

Elution conditions: 50 mM HEPES, 150 mM NaCl, 0.8 M Arginine pH 7.5

Strip conditions: 50 mM Sodium Phosphate pH 12

Flow characteristics: 50–200 cm/h (up to 2 bar)

Formulation buffer: 20% (v/v) ethanol

SKU	Product
2943932005	CaptureSelect™ Lenti VSVG Affinity Matrix 5mL
2943932010	CaptureSelect™ Lenti VSVG Affinity Matrix 10mL
2943932050	CaptureSelect™ Lenti VSVG Affinity Matrix 50mL

* *Products are Research Use Only*



ViralSEQ™ Lentivirus Physical and Proviral DNA Titer Kits

Simplify analytical development for lentiviral vector production or LV-based cell therapy

Applied Biosystems™

ViralSEQ™ Lentivirus Physical Titer Kit

A highly sensitive, robust and easy-to-use qPCR assay that is more reproducible than commonly used non-PCR based methods, with better specificity than other on-market PCR assays

Applied Biosystems™

ViralSEQ™ Lentivirus Proviral DNA Titer Kit

An easy-to-use qPCR assay as part of a complete solution to quantitate integrated proviral DNA titer in transduced cells, with high sensitivity and reproducibility

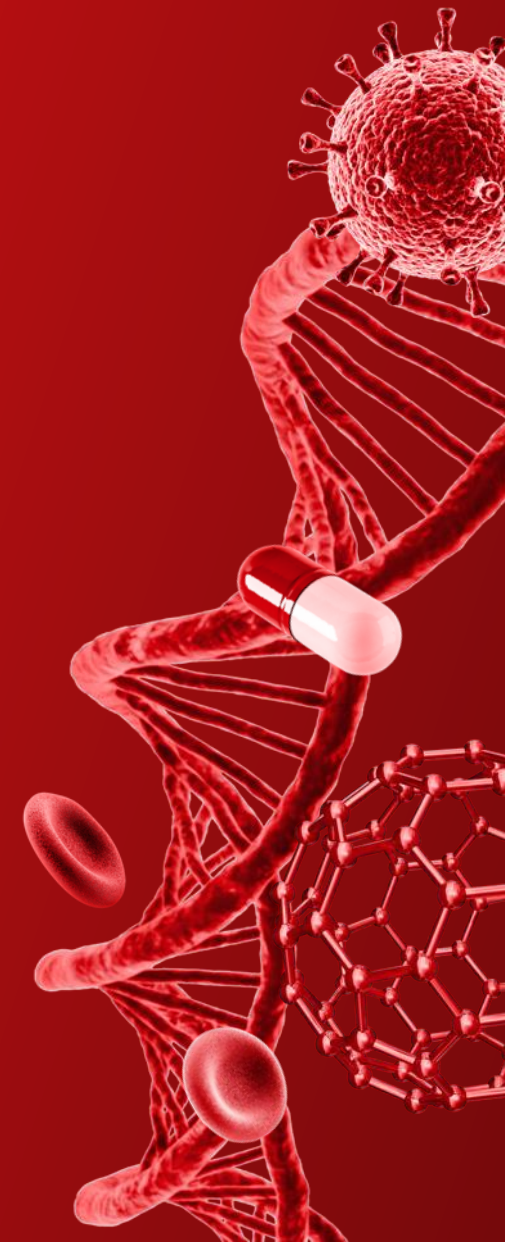


SKU	Product
A52597	ViralSEQ™ Lentivirus Physical Titer Kit
A52598	ViralSEQ™ Lentivirus Physical Titer Kit with PrepSEQ™ Nucleic Acid Sample Preparation Kit
A53561	ViralSEQ™ Lentivirus Proviral DNA Titer Kit
A53562	ViralSEQ™ Lentivirus Proviral DNA Titer Kit with PrepSEQ™ Nucleic Acid Sample Preparation Kit

Thank you

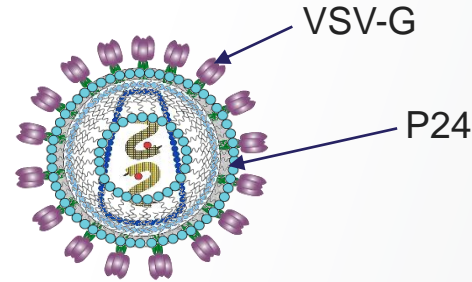
For Research Use or Further Manufacturing. Not for use in diagnostic procedures

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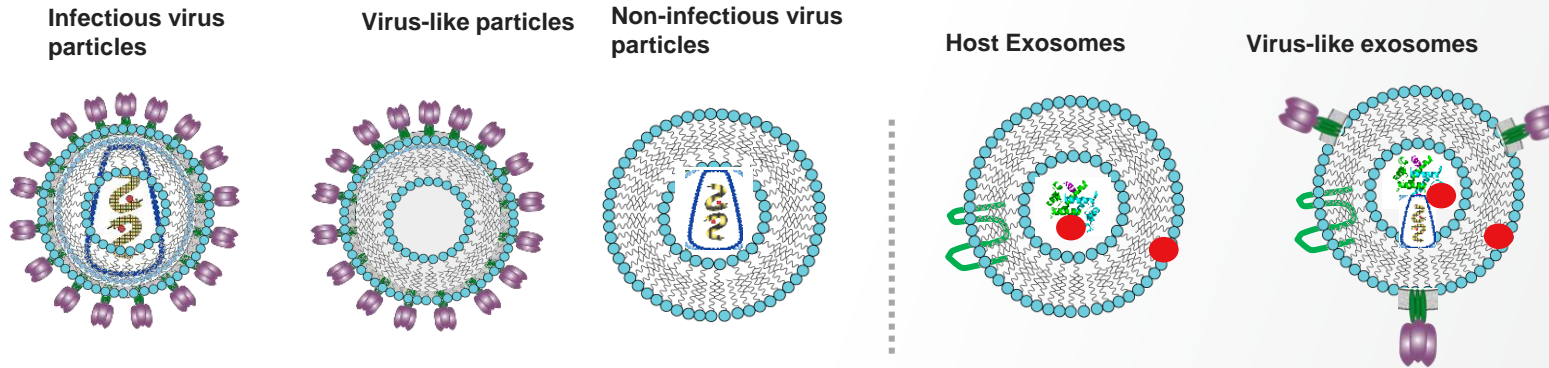


Lentivirus purification challenges

- Enveloped virus particles



- Produced in human cells (HEK293) → resulting in a broad variety of product related contaminants



- Lack of assays to discriminate / specifically detect all different forms
- **P24 ELISA**: total particles (TP) analysis, the standard for Lentivirus particles
- **IP assay**: Cell based assay to measure infectivity
- **TP/IP ratio**: Indication of quality of the Lentivirus prep; the lower the ratio the better it is.

Lentivirus purification challenges

- LVV requires a very narrow range of pH, temperature, shear stress, salt concentration, and osmolarity
- No specific affinity chromatography method commercially available
- Current methods;
 - (Ultra-)centrifugation
 - Tangential Flow Filtration TFF
 - Heparin resin, e.g. POROS 50 HE Heparin Affinity resin
 - AEX resin e.g. POROS 50 D Weak Anion Exchange Resin
 - AEX membranes e.g. Sartobind Q, Mustang Q
 - AEX nanofibers
 - Monoliths, CIM DEAE column
- Current recoveries ~30%

