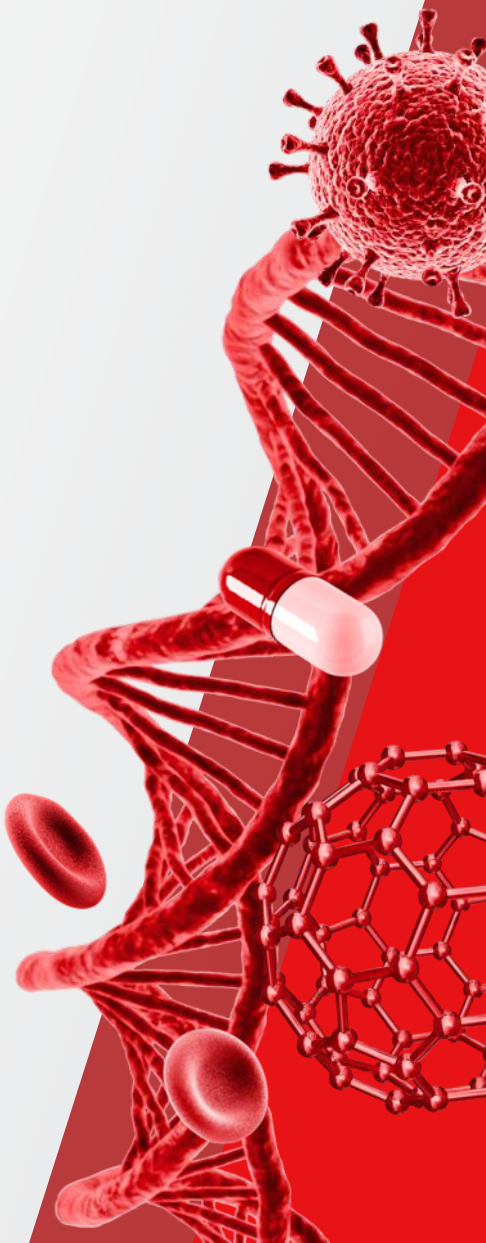
CaptureSelect™ Lenti VSVG Affinity Matrix (RUO)

Product Launch July 2022 – Update Sept 2022



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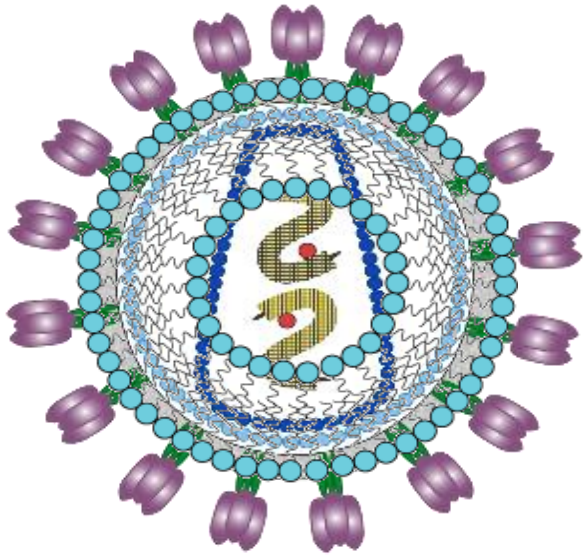
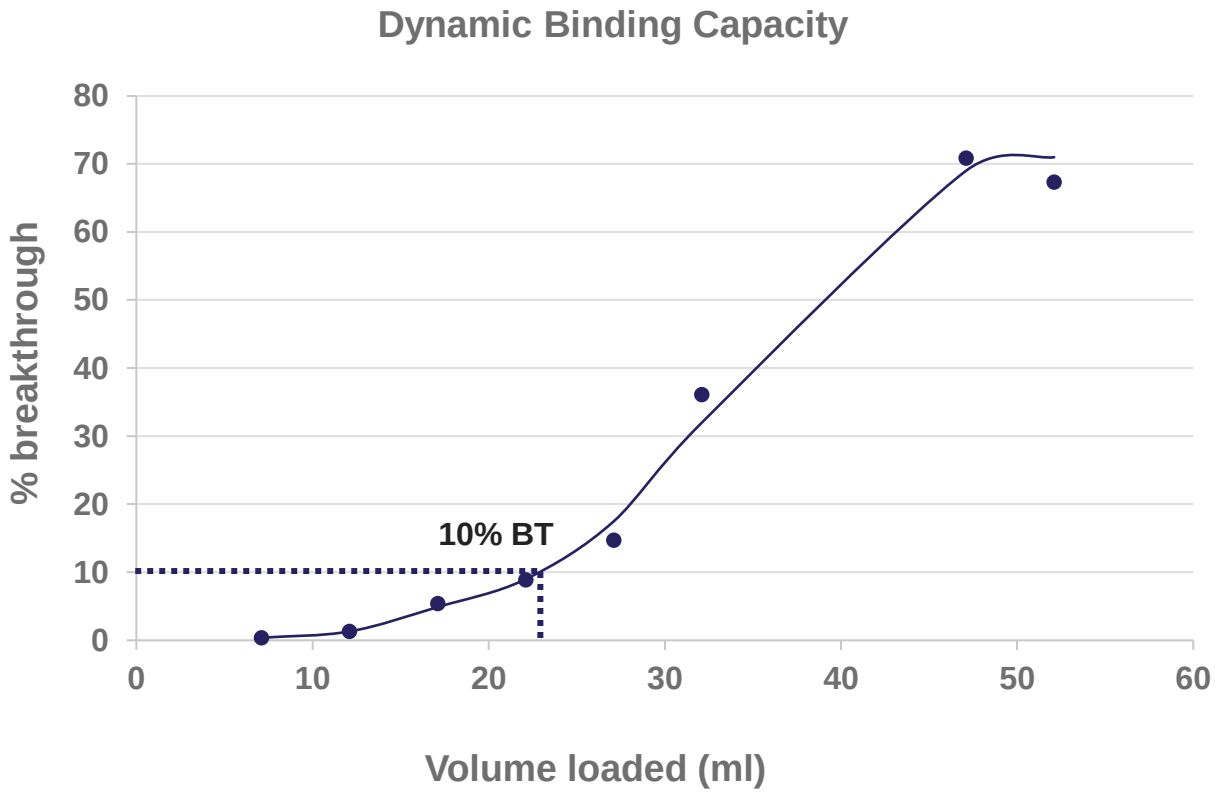
CaptureSelect Lenti VSVG affinity matrix

- 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



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

↓

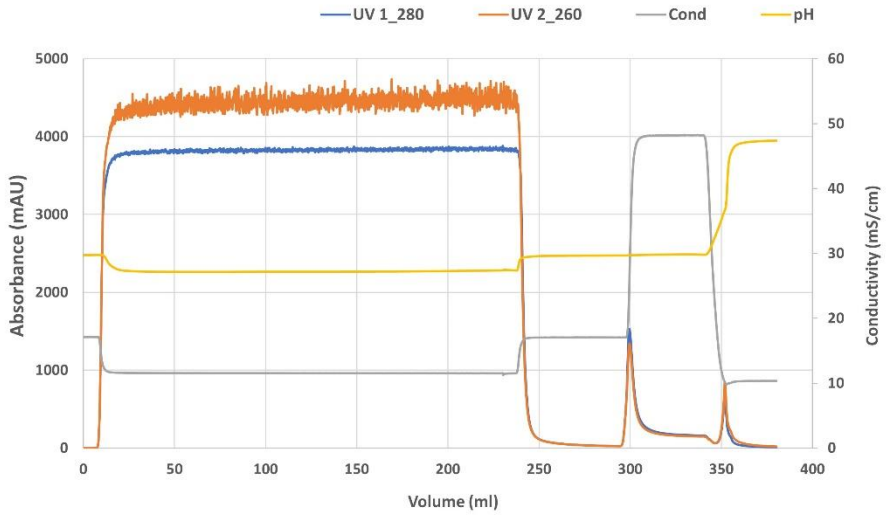
LV clarification using microfiltration (0.4 µm)

↓

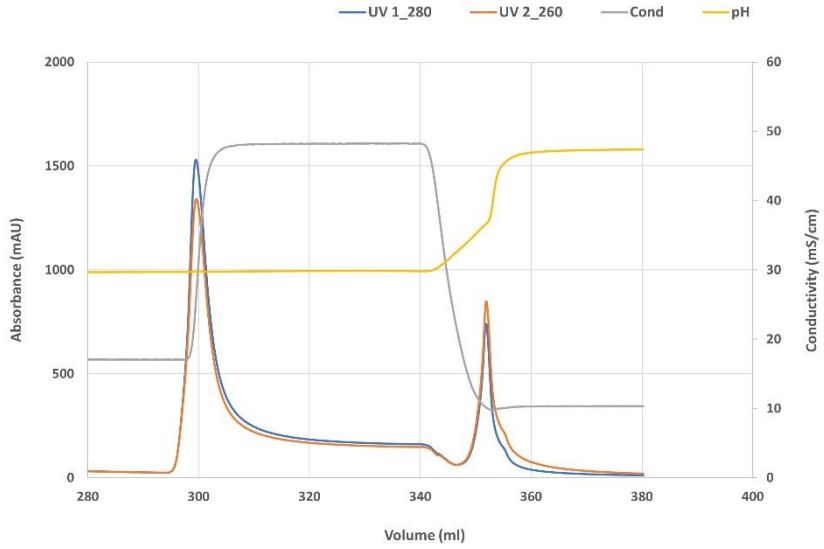
CaptureSelect Lenti VSVG affinity matrix

- 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

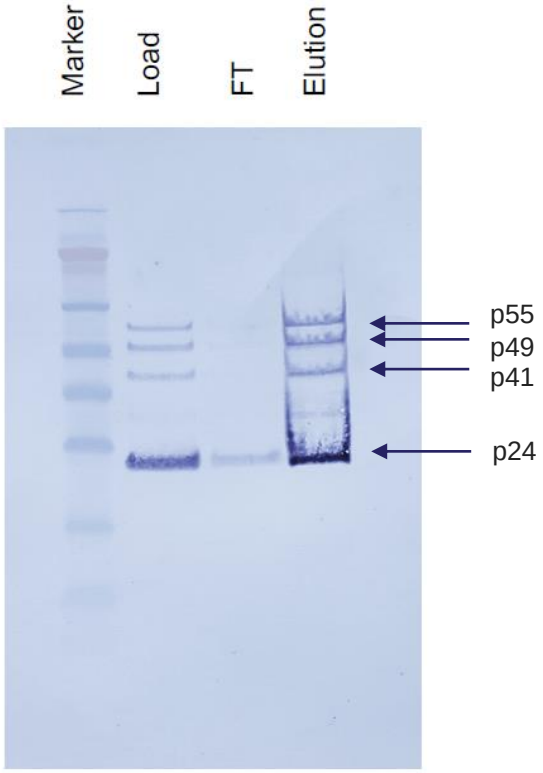


Elution close-up

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

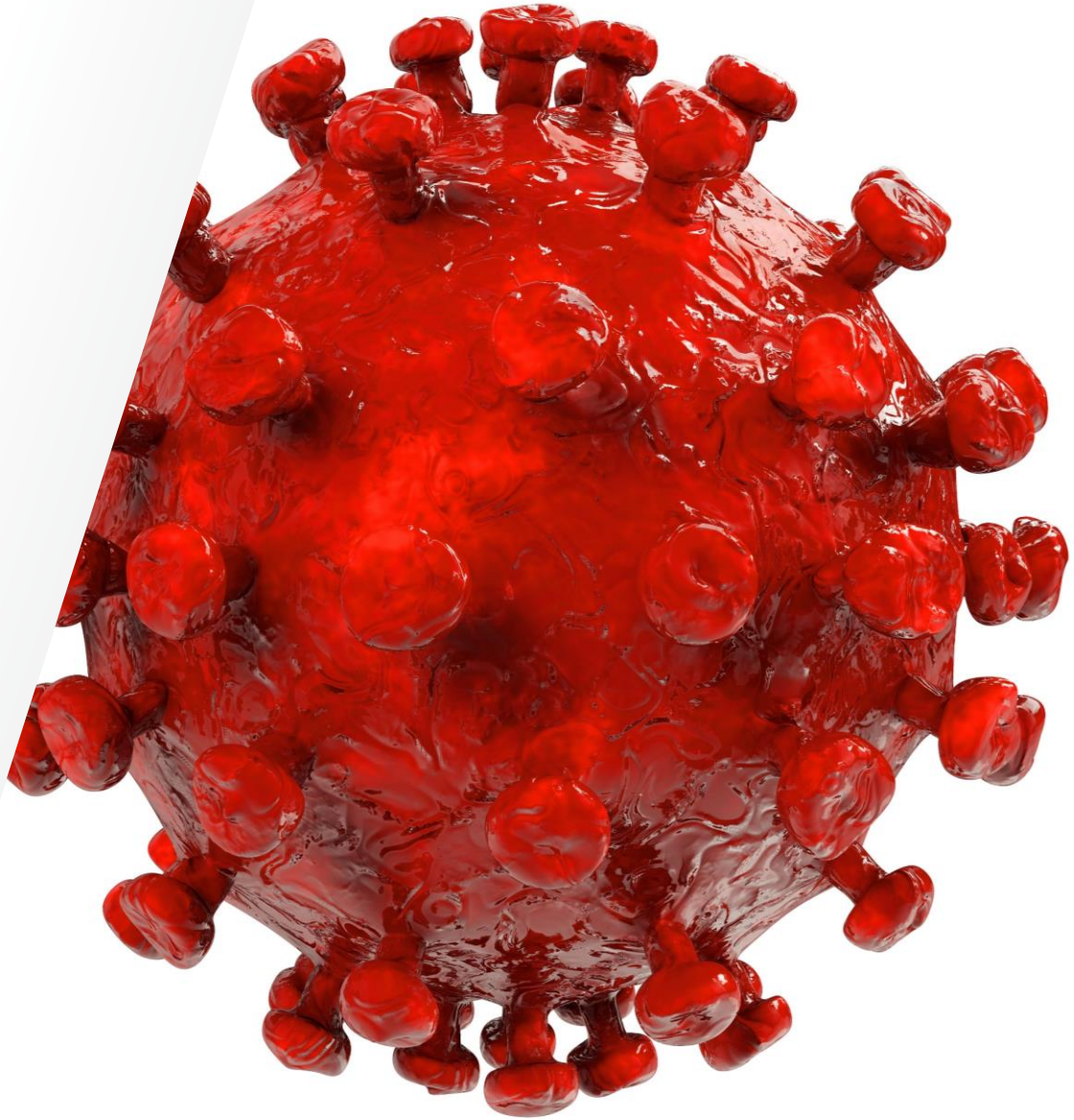
The concentration of infectious particles in the elution fraction has been enriched through purification using the Lenti-VSVG resin

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



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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 ± 10 µm
Ligand: CaptureSelect Lenti VSVG affinity ligand
Ligand coupling method: epoxide
Binding capacity: ~1E11 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

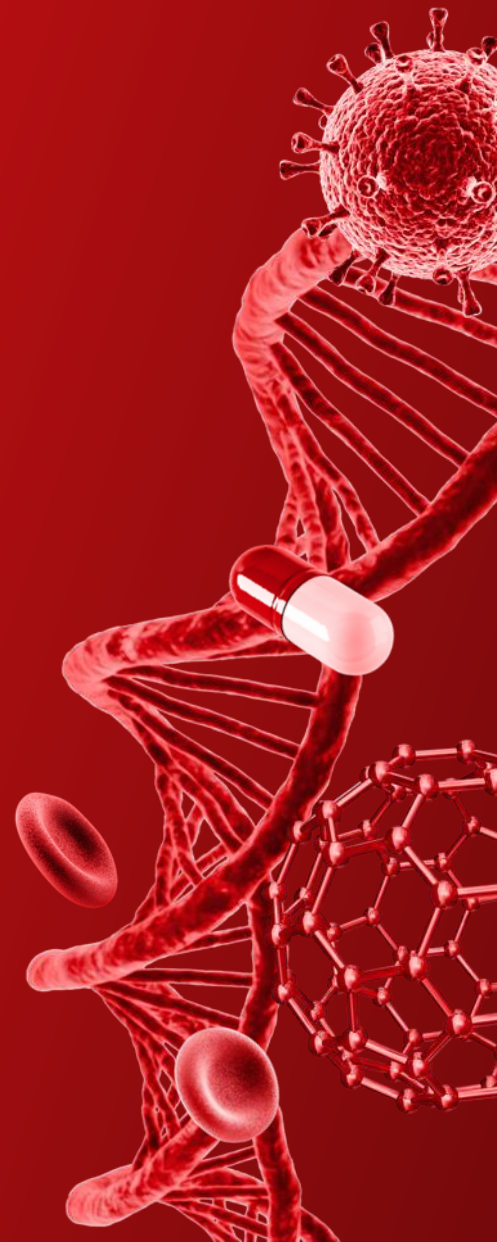
Applied Biosystems™ ViralSEQ™ Lentivirus Proviral DNA Titer Kit

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

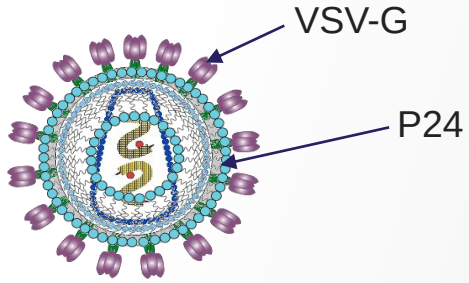
Thank you

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

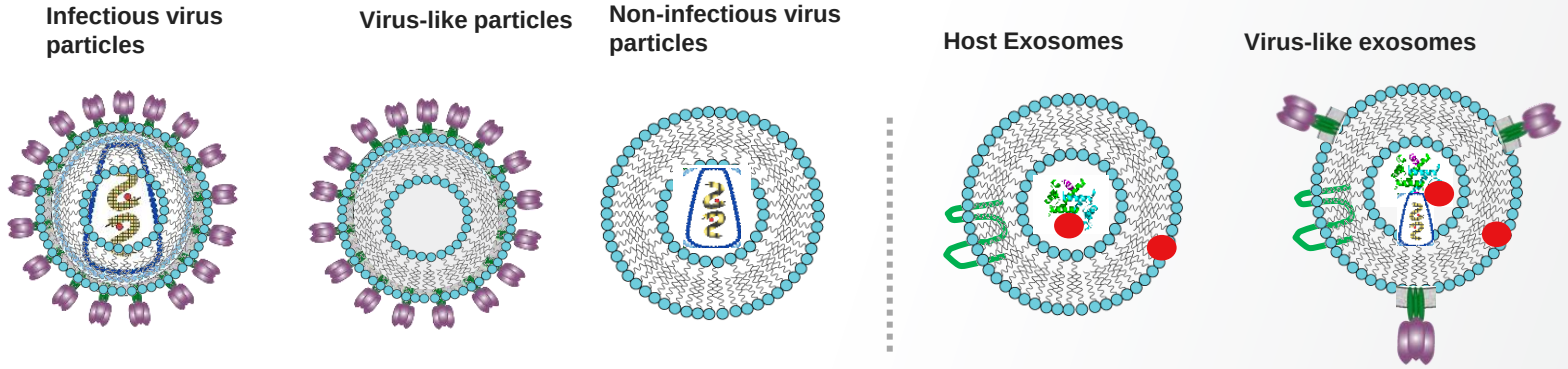


Lentivirus purification challenges

- Enveloped virus particles



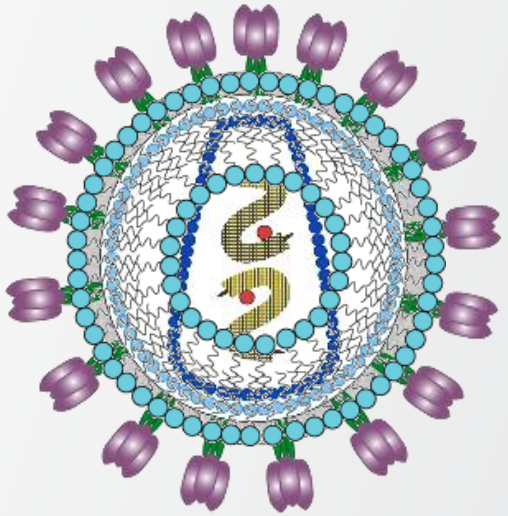
- Produced in human cells (HEK293) → resulting in a broad variety of product related contaminants



- 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%