Using affinity chromatography for the purification of Lentiviral particles

Pim Hermans, Frank Detmers, Sandra Bezemer & Laurens Sierkstra
Thermo Fisher Scientific, Leiden, the Netherlands

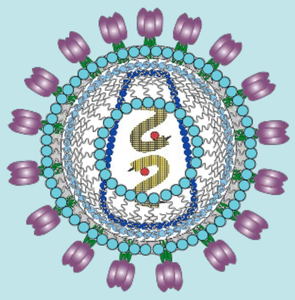
Introduction

Cell therapy vectors derived from lentiviruses offer many potentially unique advantages over more conventional retroviral gene delivery systems. Most important is their ability to provide long-term and stable gene expression and to infect non-dividing cells, such as neurons. The development pipeline of lentiviral particle-based therapies is growing and so is the need for efficient and productive production tools

Here we present a new affinity chromatography resin, the CaptureSelect™ Lenti VSVG affinity matrix, specifically designed for the purification of VSV-G pseudotyped lentivirus particles from suspension cultivations. The resin offers an efficient and scalable purification method for lentiviral particles in combination with gentle elution conditions to retain viral infectivity.

CaptureSelect Lenti VSVG affinity matrix

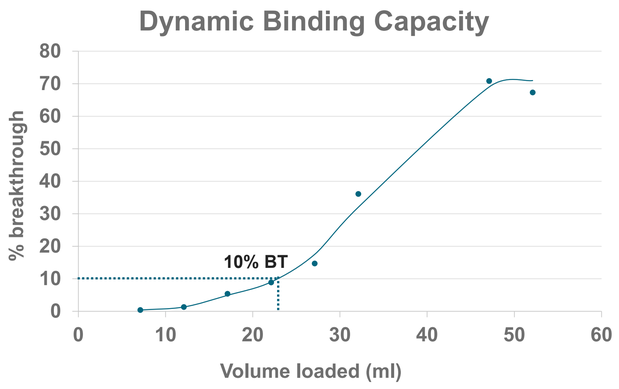
- **Based on CaptureSelect™ single-domain antibody technology**
- **Designed to bind VSV-G pseudotyped Lentiviral vector particles**
- **High recovery and purity in a single step**
- **Gentle elution conditions, based on Arginine, to retain infectivity of the lentivirus particles**
- **A scalable affinity 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

Resin performance

Dynamic Binding Capacity (DBC) study – 1mL column



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	24.6 mL
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	

Figure 1. DBC of the CaptureSelect Lenti VSVG affinity resin, determined by P24 total particle ELISA. Lentivirus produced in HEK293 cells in suspension was loaded on a 1 ml (0.66x3 cm) column, equilibrated in 50 mM HEPES, 150 mM NaCl pH 7.5. The load material had a titer of $3.89E^9$ total particles/ml. The flow-through fractions were analyzed in a p24 ELISA to determine the breakthrough of the Lentivirus particles. 10% breakthrough of the Lentivirus particles was reached after loading 24.6 ml of the feed material, resulting in a DBC of the resin of $1E^{11}$ total particles/ml of resin. C_0 is the titer of the feed stock ($3.89E^9$ particles/ml), and C is the titer measured in the flow through fractions, the 10% breakthrough point was interpolated from the breakthrough curve.

✓ **DBC at 10% breakthrough is 1E11 particles/mL resin**

- 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)

Chromatography conditions – elution profile

Process

Endonuclease treatment

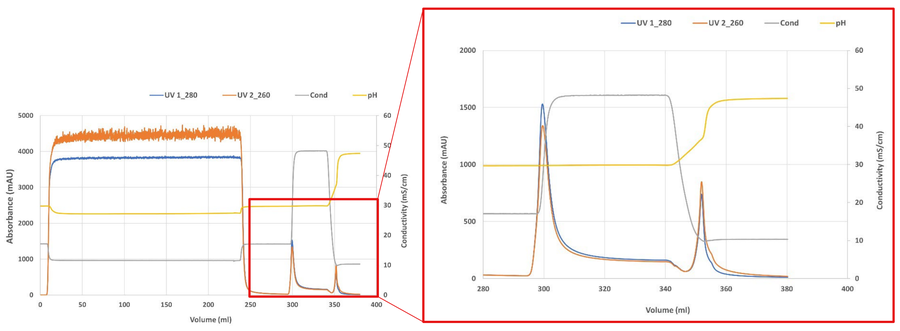
↓

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



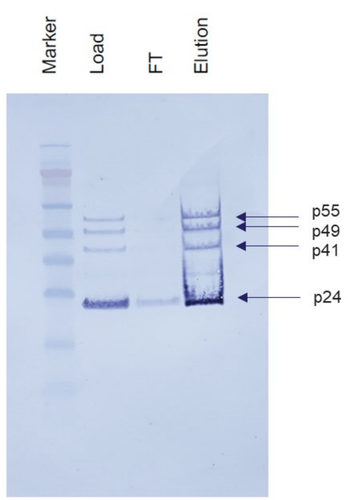
Chromatographic profile — Elution profile

Figure 2. Chromatography conditions using a 10mL column and 250 mL clarified suspension harvest with a titer of 1E10 total particles/mL
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

✓ **The resin demonstrates an efficient elution profile**

Comparison of total particle to infectious particle ratios (n=2)

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**

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

Table 1. Total particles and infectious particle ratio. Total particles are determined by p24 ELISA, infectious particles are determined through a cell infectivity assay. The data demonstrates an enrichment of infectious particles after affinity purification.

✓ **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%**

Table 2. Recovery of infectious particles determined through % of transduction units in the feed versus the elution fraction. HCP removal was measured using ELISA, total DNA was measured using Picogreen™ fluorescent probe.

✓ **Recovery of infectious particles after purification using the Lenti VSVG resin is ~50-60%**

Resin Characteristics

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

Cat. Nr.	Product
2943932005	CaptureSelect™ Lenti VSVG Affinity Matrix 5mL
2943932010	CaptureSelect™ Lenti VSVG Affinity Matrix 10mL
2943932050	CaptureSelect™ Lenti VSVG Affinity Matrix 50mL

Intended use is for Research Use Only

Conclusions

The CaptureSelect Lenti VSVG affinity matrix can be used for efficient purification of Lentivirus particles, pseudotyped with VSV-G. It is the first affinity chromatography resin available for Lentivirus purification, offering high recovery and purity in a single capture step, without compromising infectivity of the lentivirus particles.

Acknowledgements

This work has been supported by iBET Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal with a special thanks to Ana Sofia Moreira and Cristina Peixoto.