CELL & GENE THERAPY INSIGHTS

LIVE30 TRANSCRIPT

Advancing the purification of VSV-G pseudotyped lentiviral vectors by using affinity chromatography

Pim Hermans & Frank Detmers

Cell and gene therapy vectors derived from lentivirus (LV) offer unique advantages over more conventional retroviral gene delivery systems. Considering the ability to integrate the host cell genome, LV vectors have become effective tools to transduce both dividing and non-dividing cells, thereby providing long-term stable gene expression. With a growing pipeline of LV particle-based therapies comes a prominent need for more efficient manufacturing processes that are meeting the demand of functional LVs required for clinical trials. Despite the manufacturing process improvements achieved over recent years, current unit operations are still unable to reverse the significant loss of biological LV particles during the downstream process. One of the major challenges has been the development of a truly selective affinity chromatography resin that can bind the viral envelope and simultaneously allow the preservation of its biological activity during elution. This article describes a new affinity resin, suitable for the purification of VSV-G pseudotyped lentivirus particles.

Cell & Gene Therapy Insights 2023; 9(4), 389–397

DOI: 10.18609/cgti.2023.059

LENTIVIRUS PURIFICATION CHALLENGES

With the elevated use of lentivirus (LV) vector-based therapies in clinical trials, there is an increasing demand for good quality, highly pure vectors. Nevertheless, there is a plethora of purification challenges to overcome in order to reach the desired purity levels needed for clinical use.

LV is an enveloped virus that is produced using mammalian cell lines, such as HEK293 cells. One of the major challenges process developers face is separating LV vectors from

the large variety of closely-related product forms in the feedstock – for instance, exosomes. LV particles and extracellular vesicles such as exosomes follow a similar expression route in the cell. Consequently, the production of LV vectors yields a number of variations on both the vector and the exosomes, which is illustrated in Figure 1. These product-related impurities need to be removed from the final product.

A further challenge is the separation of particles with and without a genetic payload. Besides a very complex feedstock, LV vectors are relatively unstable. Therefore, sheer stress, high salt concentrations, and high osmolarity should all be avoided. In addition, only a narrow range in pH and temperature can be used when handling these particles.

The combination of these factors makes finding a suitable and efficient purification strategy challenging. Current processes report total recoveries of approximately 30% or less.

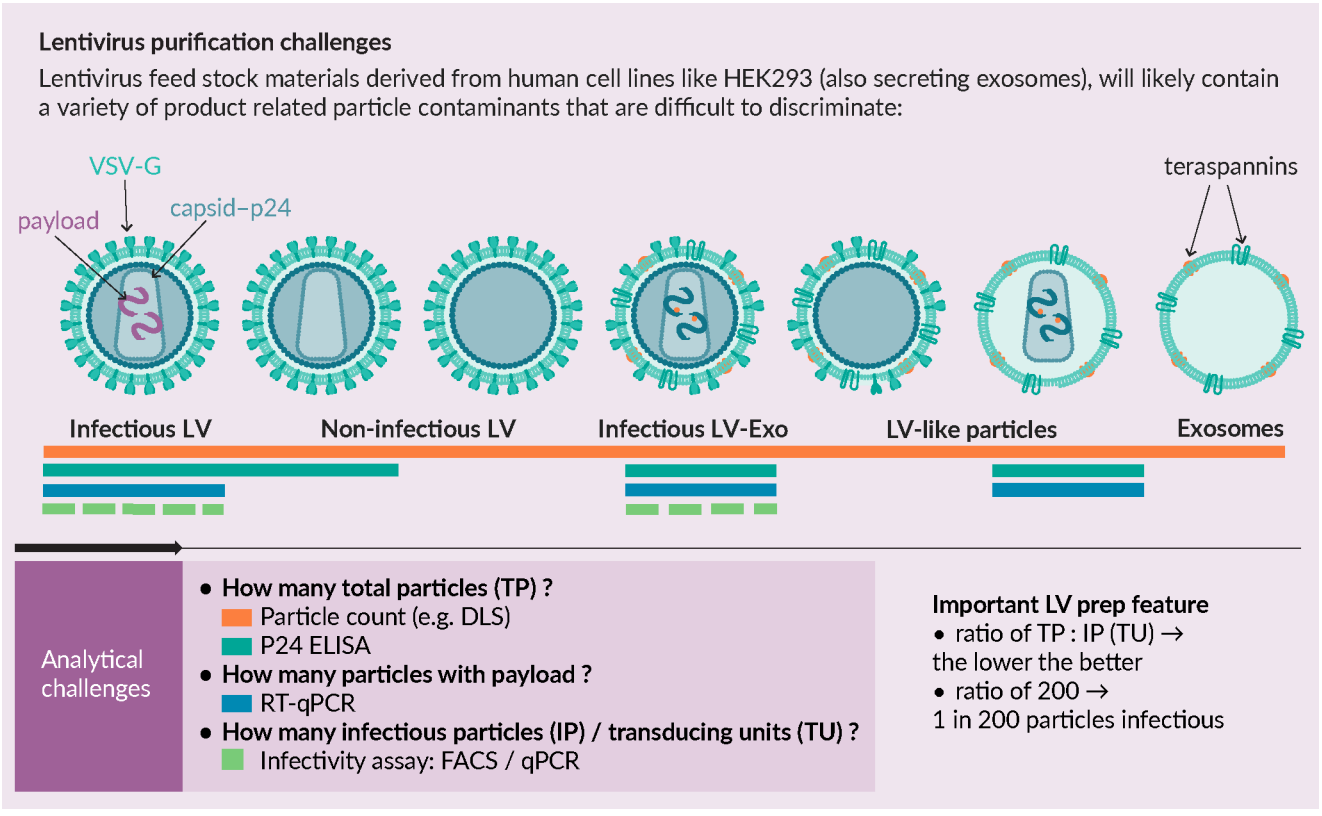
To determine both the quality of the feedstock and required steps of the purification process, it is essential to have the correct analytics in place. Important factors are the total number of particles (TP), the amount of particles with an effective payload (IP), and the ratio between these two groups (TP:IP). Figure 1 shows an overview of the various analytical assays and how they can discriminate between the different particles present in the cell culture feed or purification samples.

AFFINITY RESIN DEVELOPMENT USING CAPTURESELECT™ TECHNOLOGY

To overcome the challenges in LV purification, an affinity resin targeting the VSV-G membrane protein was developed using the CaptureSelect technology and resin development process. CaptureSelect ligands are based on single-domain antibody

FIGURE 1

Overview of lentivirus purification challenges and the analytics associated with process development.



DOI: 10.18609/cgti.2023.059

technology. The ligands are developed using an extensive screening technology where final process conditions are already implemented during screening. Ligands are tested for specificity, mild elution conditions, and stability to allow use in chromatography processes. The final ligand is recombinantly expressed in a yeast production process, which is free of animal components. CaptureSelect products are used in late clinical-stage and commercial processes. Resins are developed in a variety of drug development areas such as antibodies, biosimilars, plasma proteins, and viral vectors. The preferred resin features for the Lenti VSVG resin are shown in Box 1.

BOX 1

Preferred design features for the design of the CaptureSelect VSVG affinity matrix.

High purity and yield in a single capture step

- Good HCP and DNA clearance
- Reducing the number of purification steps
- Suitable for cell clarified harvest (no concentration)

Target release under mild elution condidions to retain LV infectivity

- Good recoveries of active LV paricles
- Improved TP:IP ratios

Scalable

Firstly, a library was created to identify binders to the VSV-G target protein. Secondly, ligands capable of binding the target were screened using a Surface Plasmon Resonance (SPR) array-based system to monitor the selectivity and the ability to release under mild elution conditions. Three ligands demonstrated good binding in the SPR assay and selectivity was confirmed using a non-related ligand binding to AAV (Figure 2, left). In addition, a concentration of 0.8 M Arginine at neutral pH was identified as a compatible

FIGURE 2

Ligand evaluation experiments showing SPR binding curves at different LV doses (left) and release efficiency using a mixed set of buffers and elution conditions (right).

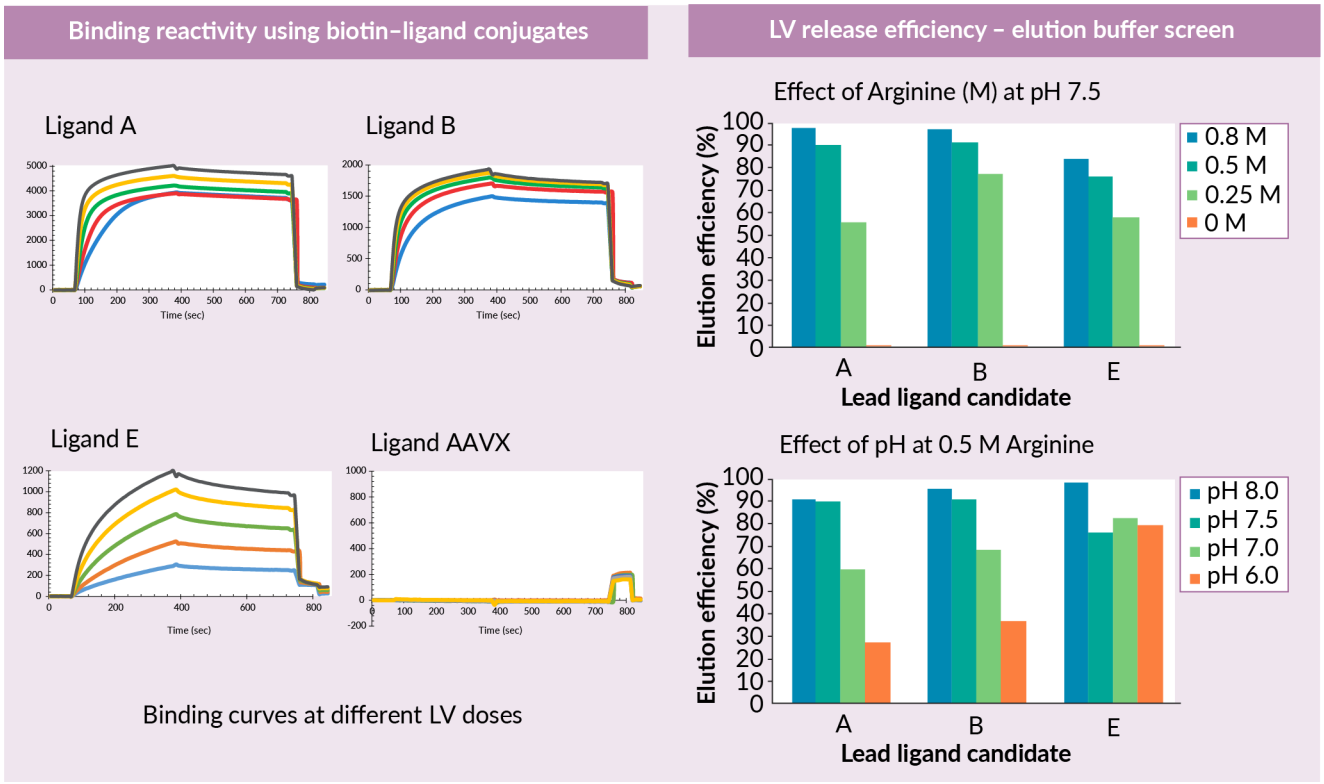
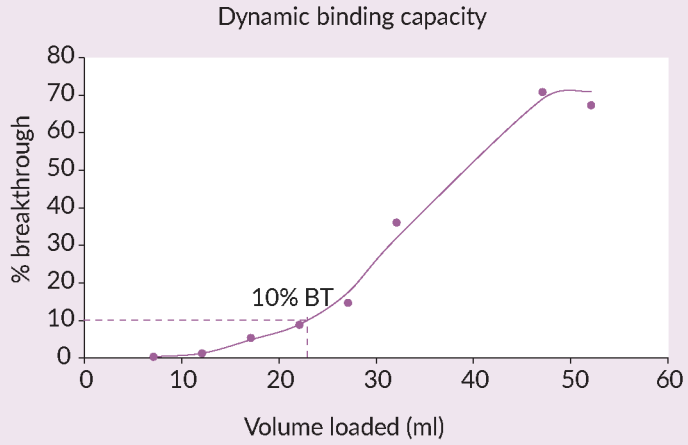


FIGURE 3

Graph showing DBC of the resin. 10% breakthrough is estimated at 1×10^{11} particles/mL.



DBC is determined by P24 total particle ELISA

Fraction	Volume (mL)	TP/mL	C/C_0
Start		3.98×10^{9}	
1	7.1	1.55×10^{7}	0.39
2	12.1	5.24×10^{7}	1.32
3	17.1	2.14×10^{8}	5.39
4	22.1	3.53×10^{8}	8.88
5	27.1	5.85×10^{8}	14.7
6	32.1	1.44×10^{9}	36.09
7	47.1	2.82×10^{9}	70.84
8	52.1	2.68×10^{9}	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 (= 1×10^{11})

elution buffer for VSV-G pseudotyped LV vectors (Figure 2, right).

After screening, three ligand candidates were expressed in a yeast production system and developed into resin prototypes, using different backbones. Resin prototypes were tested extensively in a small-scale chromatography set-up in order to determine a small selection of lead candidates for final resin development.

LENTI-VSVG RESIN CHARACTERISTICS

Dynamic binding capacity (DBC) of the resin was determined using the p24 total particle ELISA. Results are shown in Figure 3. A feed containing 4×10^{9} total particles/mL was loaded onto a 1 mL column and flow-through fractions were analyzed. Based on the results, a binding curve was plotted and the 10% breakthrough point was determined. These results show that the DBC of the resin is 1×10^{11} total particles/mL resin.

Next, purification conditions were determined in two consecutive runs on a 10 mL chromatography column, using 200 mL load material, a flowrate of 150 cm/h, and 2 min contact time. The feed was endonuclease treated, followed by a clarification on a 0.4 µm filter and direct loading on the column. Column equilibration was performed using a 50 mM HEPES buffer at pH 7.5, containing 150 mM NaCl. Elution was performed using the same HEPES buffer containing 0.8 M Arginine. After the run, a strip of the column was performed using 50 mM sodium phosphate pH12. The chromatographic profile and a close-up of the elution peak are presented in Figure 4.

Fractions of the two chromatography runs were further analyzed to determine the ratio of total particles versus infectious particles. The results, demonstrated in Table 1, reveal a five-fold enrichment of the infectious

TABLE 1

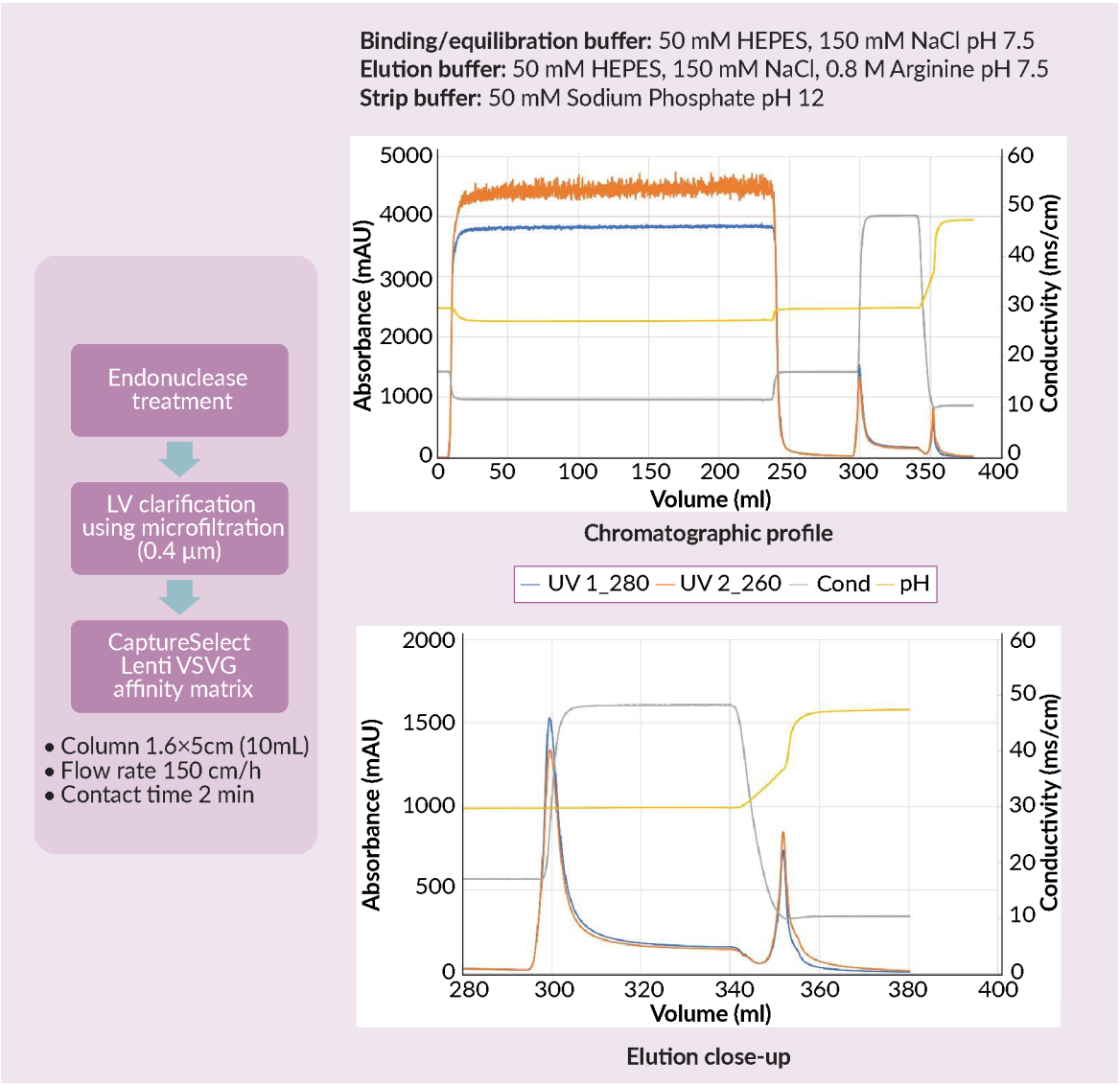
Overview of total particles and infectious particles, and their ratio (TP:IP).

Sample	TP/mL	IP/mL	TP/IP ratio
1. Feed	1.10×10^{10}	7.98×10^{7}	138
1. Flow through	3.25×10^{8}	8.30×10^{5}	392
1. Elution	4.44×10^{10}	4.42×10^{8}	100
2. Feed	1.11×10^{10}	9.00×10^{7}	123
2. Flow through	1.28×10^{9}	5.45×10^{6}	235
2. Elution	2.6×10^{10}	4.66×10^{8}	56

DOI: 10.18609/cgti.2023.059

FIGURE 4

The chromatography process using the Lenti VSVG affinity matrix (left) and the chromatographic profile (right).



particles in the final elution fraction and a decreasing TP:IP ratio. In addition, host cell protein (HCP) and DNA removal, along with total recovery of the elution fractions, was determined. Total recovery of the LV particles was between 50–60% and HCP and DNA impurity removal was considered to be highly efficient; between 80–99% (Table 2).

CONCLUSION

The CaptureSelect Lenti VSVG affinity matrix is designed to help increase productivity and efficiency in the downstream process of VSV-G pseudotyped lentiviral vectors from suspension culture. It provides gentle elution conditions, based on Arginine, to retain infectivity of the LV particles. Furthermore, the resin is a scalable affinity purification method without animal-derived components.

TABLE 2

Final recovery, HCP, and total DNA removal from the elution fractions of two chromatography runs.

Sample	Volume (mL)	IP/mL	TU (Transduction units)	Recovery	HCP removal	Total DNA removal
1. Feed	250	7.98×10^{7}	1.99×10^{10}			
1. Flow through	258	8.30×10^{5}	2.14×10^{8}			
1. Elution	22.5	4.42×10^{8}	9.95×10^{9}	49.9%	98.7%	80.2%
2. Feed	230	9.00×10^{7}	2.07×10^{10}			
2. Flow through	240	5.45×10^{6}	1.31×10^{9}			
2. Elution	25.6	4.66×10^{8}	1.19×10^{10}	57.7%	97.1%	96.5%

ASK THE EXPERTS

Abgail Pinchbeck, Assistant Editor, BioInsights speaks to (pictured left to right) **Pim Hermans,** Director of Ligand Discovery for BioProduction Group, Thermo Fisher Scientific and **Frank Detmers,** Director of Ligand Application for CaptureSelect, Thermo Fisher Scientific

Q Can you expect performance differences between suspension and adherent cultured feedstocks?

PH: Performance differences can be expected. It depends on the quality of the material. In suspension cell feeds, the ratio of total particles versus infectious particles is quite low. In adherent cell feed stocks, where the ratio can be approximately 1000:1, the composition of the material is quite different and the number of actual infectious particles is relatively low compared to the suspension cell feeds. Purification will therefore be more challenging, even for affinity solutions.

Q Can the purity level of the elution fraction regarding host cell proteins and residual DNA be further optimized?

FD: We have seen in ongoing customer evaluations that increasing the NaCl concentration between 300–450 mM for an intermediate wash buffer before eluting can help in further reducing these types of impurities. When you implement an affinity resin, the wash conditions and elution conditions are the steps that often need some optimization.

DOI: 10.18609/cgti.2023.059

Q Does the resin also work for non-VSV-G pseudotyped lentiviruses?

PH: For the development of the resin, we focused on a specific protein that is expressed by lentivirus. In this case, the VSV-G protein was chosen, which means the resin only binds to VSV-G.

Q Are there any plans to make this research-use-only resin suitable for bioprocessing?

FD: There are plans for upscaling of the resin, making it suitable for bioprocessing. It is scheduled to be available by the end of this year. It will come together with all the support packages needed such as a ligand-leakage ELISA and a regulatory support file. In addition, we are planning to generate supplementary data.

BIOGRAPHIES

PIM HERMANS leads the ligand discovery team at Thermo Fisher Scientific. Antibodies and affinity research have been a common theme throughout his whole career. After receiving his Bachelors degree in Biochemistry he proceeded to work at Holland Biotechnology where he carried out research on the production and purification of recombinant cytokines and monoclonal antibodies. By joining Unilever-Bestfoods, Pim was one of the first scientists involved in the early development and exploration of camelid derived single domain antibodies (VHHs). He joined the Bio Affinity Company (BAC, now part of Thermo Fisher Scientific) in 2003. As head of the Ligand Discovery Department Pim is responsible for the development of VHH based affinity ligands for applications in process – and analytical affinity chromatography. Through the introduction of new selection and screening methodologies he and his team enabled rapid development of affinity purification - and detection tools serving a broad variety of targets.

FRANK DETMERS received his PhD at the department of Molecular Microbiology of the University of Groningen (The Netherlands). From 2001 until 2004 he worked as a post-doctoral researcher at the Department of Cell Physiology at the Nijmegen Center of Molecular Life Sciences (NCMLS, Nijmegen, The Netherlands). He joined BAC BV (Leiden, The Netherlands) in 2004 and the focus of his work is immobilization of affinity ligands on solid supports and the development of new applications of the CaptureSelect ligands. Currently, Frank is director of ligand application for CaptureSelect at ThermoFisher. His work is focusing on the development of new purification tools in the field of antibodies, therapeutic proteins, and gene and cell therapy.

AFFILIATIONS

Pim Hermans
Director of Ligand Discovery for BioProduction Group,
Thermo Fisher Scientific

Frank Detmers, PhD
Director of Ligand Application for CaptureSelect,
Thermo Fisher Scientific

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The authors have no conflicts of interest.

Funding declaration: The authors received no financial support for the research, authorship and/or publication of this article.

Article source: This article is a transcript of a webinar, which can be found here.

Webinar recorded: Mar 7 2023; **Revised manuscript received:** Apr 21 2023; **Publication date:** date

DOI: 10.18609/cgti.2023.059

ThermoFisher SCIENTIFIC

Pharmaceutical analytics

The way forward in lentivirus quantitation.

Rapid analytical solutions for lentiviral vector titer and characterization

Applied Biosystems™ ViralSEQ™ lentivirus titer kits are easy-to-use, robust, real-time PCR (qPCR) assays for lentivirus (LV) characterization that enable validation to meet regulatory expectations.

The Applied Biosystems™ ViralSEQ™ Lentivirus Physical Titer Kit measures genome-containing LV particles, while the Applied Biosystems™ ViralSEQ™ Lentivirus Proviral DNA Titer Kit quantifies integrated proviral DNA copies in transduced cells for infectious titer and vector copy number assessment.

These kits provide a convenient method to quantitate and correlate qPCR data for total and infectious LV particles, facilitating analytics in process development, optimization, and manufacturing quality control (QC).

Learn more at **thermofisher.com/viralseq-lentivirus**

applied biosystems