Revolutionizing Gene Therapy Protein Expression Potency Assays

Fast 96-Sample Throughput for Comprehensive, Fully Quantitative Protein Analysis with the Leo System

Precise Protein Quantitation with Size Resolution in 3 Hours

As gene therapies continue to advance, the need for scalable, reproducible, and efficient potency assays is increasing—especially for adeno-associated virus (AAV) vectors. AAVs are leading platforms for gene therapies targeting various tissues (e.g., liver, muscle, CNS, retina). Accurate quantification of transgene expression and functional protein production is crucial for meeting regulatory standards and ensuring therapeutic efficacy.[1]

Traditional AAV potency methods, such as Western blot, ELISA, and cell-based assays, present well-known challenges.[2] The **Leo™ System** powered by **Simple Western™ Technology** offers a breakthrough by enabling fully automated, quantitative, and ultra-sensitive protein analysis of up to 96 samples in a single three-hour run. Unlike conventional methods, Leo provides absolute quantification without gel electrophoresis, membrane transfer, or manual blotting. This makes it ideal for high-throughput AAV potency testing, drastically reducing both assay time and hands-on labor compared to traditional Western blot or ELISA.

Why Choose Leo for AAV Potency Testing?

High-Throughput. Process up to 96 samples in as little as 3 hours, setting a new benchmark for high-throughput Western analysis.

Universal Platform. Works with any AAV serotype or target protein, requiring only a single validated antibody for detection.

High Sensitivity & Low Sample Volume. Detects low-abundance protein expression at the picogram level and requires just 3 µL of precious sample.

Automated & Scalable. Minimizes hands-on time and reduces bottlenecks, enabling larger and more efficient studies.

Reproducible & Quantitative. Includes total protein normalization to deliver accurate, quantitative results.

Faster Turnaround. Reduces analysis time from days to hours, accelerating product development timelines.

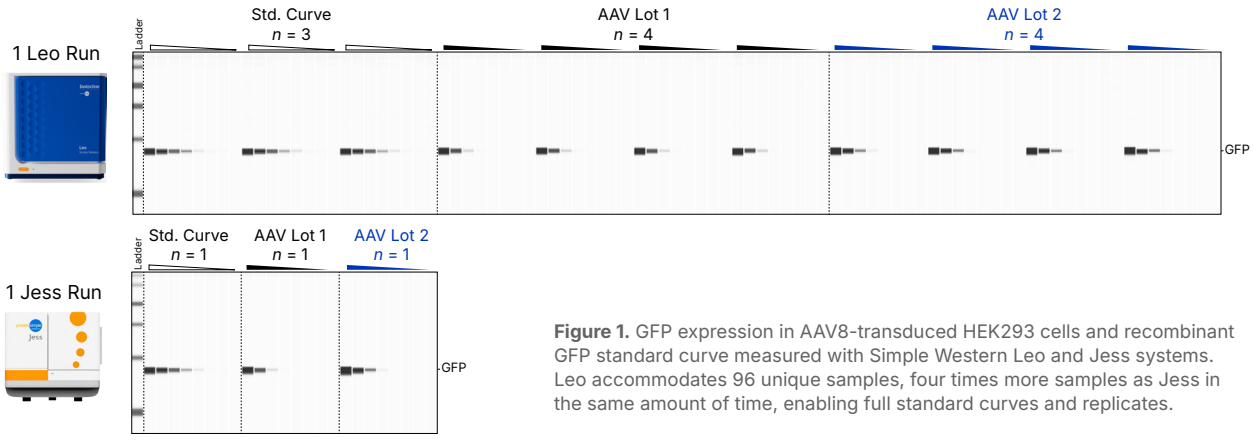

Figure 1. GFP expression in AAV8-transduced HEK293 cells and recombinant GFP standard curve measured with Simple Western Leo and Jess systems. Leo accommodates 96 unique samples, four times more samples as Jess in the same amount of time, enabling full standard curves and replicates.

Comparative AAV Potency Testing with Simple Western Leo and Jess

During AAV-based gene therapy development, ensuring batch-to-batch consistency is critical for both regulatory approval and therapeutic efficacy.[2-3] Accurate quantification of transgene expression and therapeutic protein production can reveal important lot-to-lot variations that may affect clinical outcomes.

In this study, we compared the relative potency of two AAV8 lots, examining how stress factors and storage conditions affect transduction efficiency in HEK293 cells using the Simple Western Leo and Jess systems. Both systems are fully automated, quantitative, and ultra-sensitive, offering accelerated sample processing with reproducible protein quantification. Our results correlated well with traditional methods, such as flow cytometry. Notably, Leo generated four times more data than Jess in the same amount of time (**Figure 1**), highlighting its potential for scalable, efficient potency testing.

Materials and Methods

AAV Transduction of HEK293 Cells

HEK293 cells were transduced with two AAV8 lots containing DNA encoding CMV-GFP (Virovek, 277B545). Each lot was either stressed at 37 °C for 5 days or kept as a non-stressed control. Eight MOIs were tested for each condition (0 vector genomes per cell to 3.00 × 10^6 vg/cell), for a total of 32 samples. Cells were harvested 48 hours post-transduction for analysis on flow cytometry for GFP fluorescence and live/dead staining. The remaining cell harvest was flash frozen for Simple Western analysis.

Transgenic GFP Expression Analysis using Simple Western Systems Leo and Jess

Transgenic GFP expression was analyzed using Leo and Jess Systems and the materials are listed in **Table 1**. All samples were denatured under reducing conditions in 1X Master Mix for 5 minutes at 95 °C. Milk-Free Antibody Diluent was used for blocking and for primary antibody dilution. The primary anti-GFP antibody was diluted to 20 µg/mL, and the anti-Goat secondary antibody was used at a ready-to-use concentration.

Relative Potency Measurements

Whole-cell lysates from transduced HEK293 cells were analyzed at 0.2 mg/mL. Stressed and control samples from each lot were analyzed in replicate on Leo and Jess as indicated. AAV8 control lots served as the reference material; stressed lots served as test materials. GFP expression was normalized to total protein, which was measured using RePlex with chemiluminescence detection. Parallel line analysis (PLA) determined relative potency at 3 different levels, (Y) = 4.5, 5, 5.5.[5]

Absolute Protein Quantification

A standard curve was prepared by titrating recombinant GFP in an 8-point, two-fold dilution series (20.0 ng/mL to 0.156 ng/mL) in 0.1 mg/mL non-transduced HEK293 lysate background. Whole-cell lysates from transduced cells were analyzed at 0.1 mg/mL. Stressed and control lots were tested in replicate on Leo and Jess as indicated, and the expressed GFP concentration was calculated based on the recombinant GFP standard curve.

Name	Cat. #
Jess 12-230 kDa Separation Module	SM-W001
Leo 12-230 kDa Separation Module	SWSM-W014
EZ Standard Pack 2	PS-ST01EZ-8
Anti-Goat Detection Module	DM-006
Total Protein Detection Module	SWDM-TP21
RePlex™ Module	RP-001
GFP Antibody	AF4240
Recombinant GFP Protein	NBC1-22949

Table 1. Materials used for Simple Western experiments on Leo and Jess. All materials are available from Bio-Techne.

Results

Impact of Stress on AAV Potency

Previous studies have shown that AAVs can undergo asparagine deamidation at elevated temperatures, resulting in reduced transduction.[5-7] In our study, AAV8 lots incubated at 37 °C for 5 days resulted in reduced GFP expression compared to non-stressed controls when used to transduce cells (**Figure 2**).

Both Simple Western systems revealed a 0.4–0.5-fold reduction in potency for the stressed lots (**Figure 3, Table 2–3**). These findings underscore the importance of managing storage conditions of gene therapy products and highlight the robust quantitative ability of Simple Western assays to resolve small differences in transduction efficiency as a result of stress.

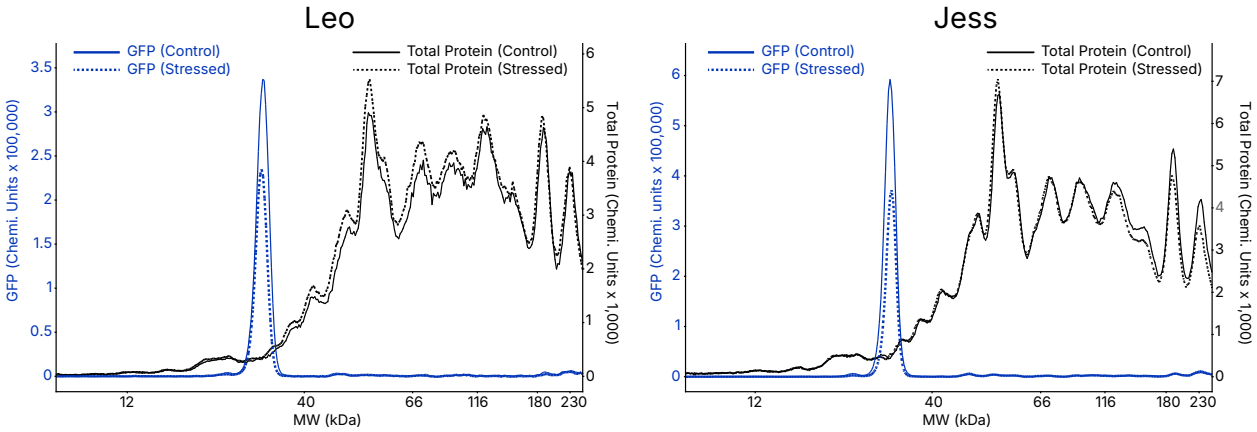

Figure 2. Analysis of AAV-mediated GFP expression in HEK293 cells. Stressed samples (dashed traces) and non-stressed controls (solid traces) were measured on Leo (left) and Jess (right). GFP expression (blue traces) and total protein (black traces) were measured in the same capillary using the RePlex. HEK293 cells were transduced with AAV8 at MOI of 3.00 × 10^6 vg/cell.

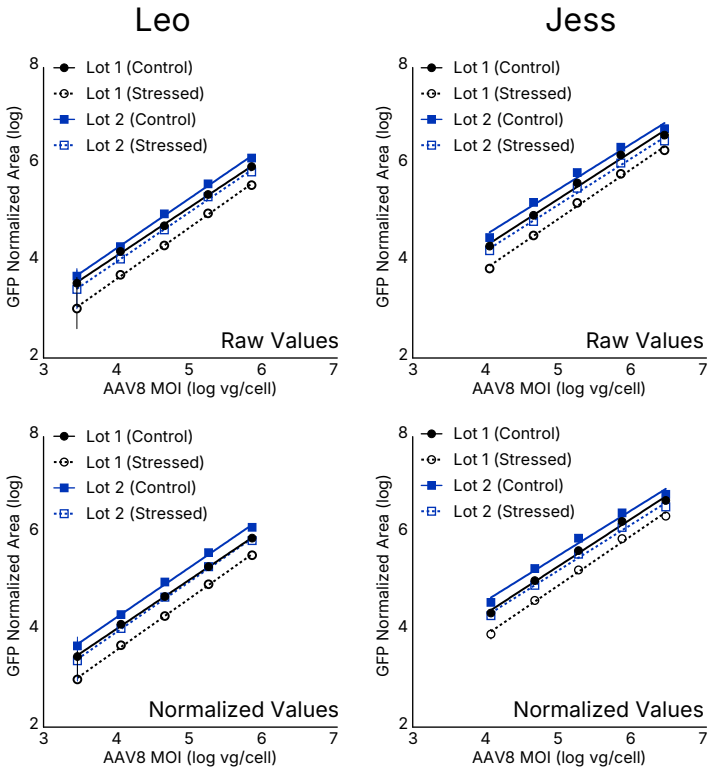

Figure 3. PLA to determine relative potency of stressed and non-stressed (control) AAV samples using Simple Western systems Leo and Jess. Values represent averages resulting from 3 replicates on Leo (*n*=3) and 2 replicates on Jess (*n*=2).

Instrument	Lot 1 Control		Lot 1 Stressed	
	Avg	% CV	Avg	% CV
Leo	1	-	0.43	5.5
Jess	1	-	0.40	5.4

Table 2. Relative potency analysis of stressed and non-stressed (control) AAV samples (lot 1) using Simple Western systems Leo and Jess. The non-stressed control was used as the reference sample. Values represent averages resulting from 3 replicates on Leo (*n*=3) and 2 replicates on Jess (*n*=2).

Instrument	Lot 2 Control		Lot 2 Stressed	
	Avg	% CV	Avg	% CV
Leo	1	-	0.52	1.2
Jess	1	-	0.47	1.7

Table 3. Relative potency analysis of stressed and non-stressed (control) AAV samples (lot 2) using Simple Western systems Leo and Jess. The non-stressed control was used as the reference sample. Values represent averages resulting from 3 replicates on Leo (*n*=3) and 2 replicates on Jess (*n*=2).

Lot-to-Lot Variability

Ensuring consistent AAV vector quality is essential for predictable clinical outcomes.[1-3] Lot-to-lot variation arises from inherent variability of starting materials and manufacturing conditions, which is complicated by the establishment of assays sensitive enough to reproducibly detect small differences in potency between lots.

To determine whether the Leo and Jess systems could detect inter-lot differences, we compared the two AAV8 lots. Both systems revealed that lot 2 was significantly more potent than lot 1, with a total protein–normalized relative potency of around 1.9, with reproducibility of CV at 6% or lower (**Figure 4**, **Table 4**). Importantly, the small sample requirements (3 µL) demonstrates that lot-to-lot variation can be consistently measured with minimal impact on final vector product concentrations.

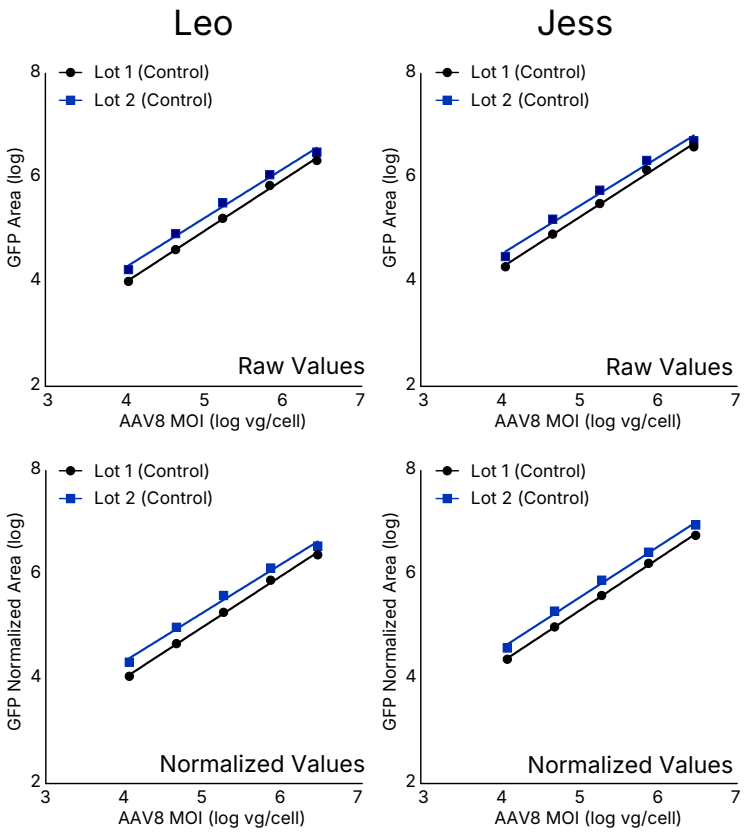

Figure 4. PLA to determine the relative potency of two lots of non-stressed (control) AAV8 samples using Simple Western systems Leo and Jess. Values represent averages resulting from 6 replicates on Leo (*n*=6) and 4 replicates on Jess (*n*=4).

	Instrument	Relative Potency	% CV
Raw	Leo	1.8	4.7
	Jess	1.8	5.7
Normalized	Leo	1.9	6.0
	Jess	1.8	2.4

Table 4. Relative potency analysis of two lots of non-stressed (control) AAV8 samples using Simple Western systems Leo and Jess. Values represent average relative potency measurements resulting from 6 replicates on Leo (*n*=6) and 4 replicates on Jess (*n*=4). Lot 1 was used as the reference.

Absolute Transgene Expression

Though many potency assays focus on relative changes, knowing the absolute amount of transgenic protein (e.g., ng/mL or fg/cell) provides more direct evidence of vector efficacy and supports better-defined acceptance criteria and dose-response assessments.[8-10] To calculate GFP concentration, we included a standard curve of recombinant GFP during analysis on both Leo and Jess, resulting in similar slopes and R^2 values (**Figure 5**). The recovery rates of the standard curves were calculated to ensure accurate GFP quantification (**Table 5**).

By running a recombinant GFP standard curve on Leo and Jess, we measured absolute protein concentrations down to 0.04 ng/mL (or 0.07 fg/cell) (**Tables 6–7**). As observed in the relative potency data, the stressed lots exhibited lower protein expression than the controls, and lot 2 outperformed lot 1.

rGFP	% Recovery	
(ng/mL)	**Leo**	**Jess**
20	100	93.5
10	99.9	94.6
5	106	103
2.5	99.8	112
1.25	99.9	103
0.625	90.0	103
0.3125	99.9	97.3
0.15625	109.9	95.4

Table 5. Recovery of GFP standard curves.

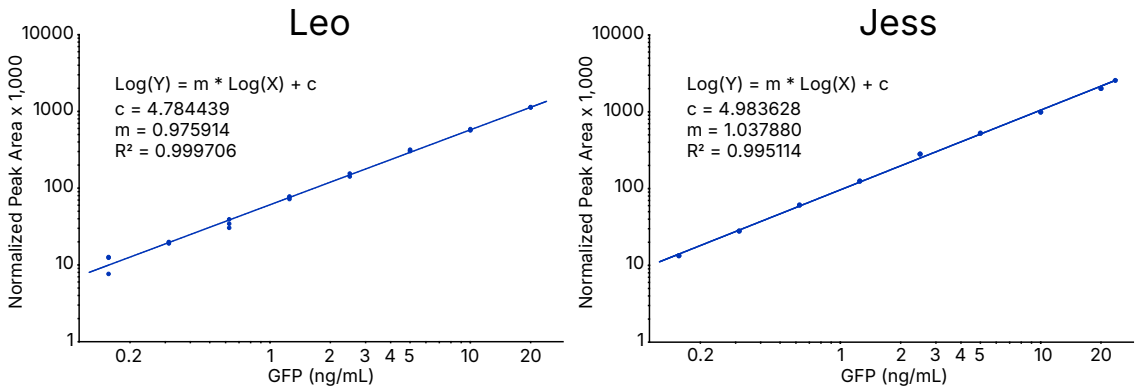

Figure 5. Standard curve of recombinant GFP for absolute quantification of AAV-mediated GFP expression using Simple Westerns systems Leo and Jess.

AAV8 (vg/cell)	Lot 1 Control		Lot 1 Stressed		Lot 2 Control		Lot 2 Stressed	
	Leo	Jess	Leo	Jess	Leo	Jess	Leo	Jess
	GFP (ng/mL)							
3.00 × 10⁶	23	24	9.3	9.4	36	45	19	22
7.50 × 10⁵	6.9	8.2	2.8	3.9	12	16	6.1	7.9
1.88 × 10⁵	1.6	2.1	0.63	0.90	3.2	4.4	1.6	2.0
4.69 × 10⁴	0.38	0.48	0.14	0.26	0.67	1.1	0.34	0.49
1.17 × 10⁴	0.08	0.14	0.04	0.05	0.15	0.24	0.07	0.10
	GFP (fg/cell)							
3.00 × 10⁶	53	54	22	22	79	99	31	37
7.50 × 10⁵	12	14	6.6	9.3	20	27	12	16
1.88 × 10⁵	2.7	3.6	2.0	2.9	4.7	6.4	2.4	3.0
4.69 × 10⁴	0.54	0.70	0.39	0.71	1.5	2.5	0.50	0.72
1.17 × 10⁴	0.13	0.21	0.07	0.09	0.26	0.41	0.10	0.13

Table 6. Absolute quantification of GFP expression in HEK293 cells transduced with 2 lots of stressed and non-stressed (control) AAV8 samples. Values were derived from the standard curve and represent averages resulting from 2 replicates on Leo (*n*=2) and 1 replicate on Jess (*n*=1).

AAV8 (vg/cell)	Run 1				Run 2			
	Lot 1 Control		Lot 2 Control		Lot 1 Control		Lot 2 Control	
	Leo	Jess	Leo	Jess	Leo	Jess	Leo	Jess
	GFP (ng/mL)							
3.00 × 10⁶	23	24	36	45	28	29	45	43
7.50 × 10⁵	6.9	8.2	12	16	7.8	9.0	15	15
1.88 × 10⁵	1.6	2.1	3.2	4.4	1.7	1.9	3.5	3.8
4.69 × 10⁴	0.38	0.48	0.77	1.1	0.41	0.45	0.8	1.0
1.17 × 10⁴	0.08	0.14	0.15	0.24	0.08	0.09	0.17	0.20
	GFP (fg/cell)							
3.00 × 10⁶	53	54	79	99	64	65	100	94
7.50 × 10⁵	12	14	19	28	13	16	25	26
1.88 × 10⁵	2.7	3.6	4.7	6.4	2.9	3.2	5.1	5.5
4.69 × 10⁴	0.54	0.70	1.5	2.5	0.59	0.65	1.8	2.4
1.17 × 10⁴	0.13	0.21	0.26	0.41	0.12	0.14	0.29	0.34

Table 7. Absolute quantification of GFP expression in HEK293 cells transduced with 2 lots of non-stressed (control) AAV8 samples. Values were derived from the standard curve and represent averages resulting from 4 replicates on Leo (*n*=4) and 2 replicates on Jess (*n*=2). Two separate runs are shown to demonstrate assay robustness and data quality. While Run 1 showed a difference between Leo and Jess of approximately 9 ng/mL in the lot 2 control samples at the highest MOI (3.00 × 10^6 vg/cell), Run 2 showed very similar concentrations between instruments.

Comparison to Flow Cytometry

Measuring both functional protein output and absolute protein levels establishes a clearer relationship between AAV genome delivery and actual therapeutic protein production.[10] Consistent with previous observations, analysis by flow cytometry revealed stressed AAV samples were less efficient at transduction compared to non-stressed controls (Figure 6).

Further validation was conducted by comparing Simple Western data with flow cytometry data. Simple Western and flow cytometry results showed a strong correlation (**Figure 6**), further supporting the reliability and consistency of Leo for protein expression potency testing.

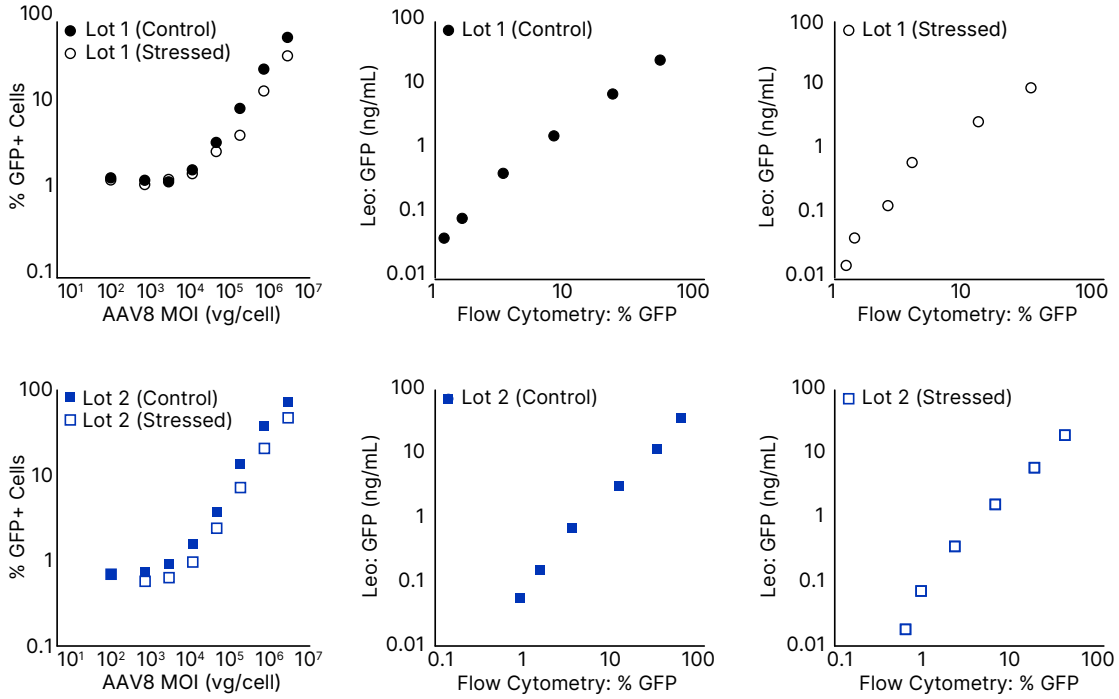

Figure 6. Comparison of Simple Western and flow cytometry analysis of transgenic GFP expression in AAV-transduced HEK293 cells. (**Left Panels**) Flow cytometry analysis of HEK293 cells 48 hours post-transduction with stressed and non-stressed (control) AAV8 samples. (**Middle and Right Panels**) Absolute GFP concentrations in HEK293 cells measured using Simple Western plotted against %GFP cells measured using flow cytometry.

Conclusion

The Simple Western Leo system is a powerful, high-throughput platform for AAV potency testing, offering distinct advantages over traditional methods in throughput, sensitivity, and reproducibility. By processing 96 samples in as little as 3 hours, Leo provides rapid, quantitative, and reliable data ideally suited to both early-phase research and batch-release testing of gene therapy products.

Key findings from this application note include:

- **Robust Detection of Stress-Induced Potency Changes:** Leo clearly detects lower potency in AAV8 lots exposed to a 5-day stress test at 37 °C.
- **Accurate Lot-to-Lot Comparisons:** Leo can reliably distinguish potency differences between lots, supporting quality control efforts and regulatory requirements.
- **Relative and Absolute Quantification:** Ability to measure both relative potency (via PLA) and absolute protein levels (using a recombinant standard curve) in a single workflow.
- **Strong Correlation with Other Methods:** Data align well with Jess system results and flow cytometry, underscoring Leo's reliability.

These attributes make Simple Western Leo an excellent choice for accelerating and strengthening AAV-based gene therapy development. With its ability to analyze large sample sets rapidly and quantitatively, Leo can help researchers and manufacturers meet the rigorous demands of regulatory bodies while driving innovations in gene therapy.

References

1. U.S. Food and Drug Administration. (2023). Final guidance for industry: Potency tests for cellular and gene therapy products. U.S. Department of Health and Human Services. Link.
2. Fu Q, Polanco A, Lee YS, Yoon S. Critical challenges and advances in recombinant adeno-associated virus (rAAV) biomanufacturing. Biotechnol Bioeng. 2023 Sep;120(9):2601-2621.
3. O'Connor DM, Lutomski C, Jarrold MF, Boulis NM, Donsante A. Lot-to-Lot Variation in Adeno-Associated Virus Serotype 9 (AAV9) Preparations. Hum Gene Ther Methods. 2019 Dec;30(6):214-225.
4. McElroy W, et al. Enabling icIEF Peak Identification of AAV Capsid Proteins by Fractionation on MauriceFlex and Subsequent Analysis by LC-MS. Electrophoresis. 2025 Jan;46(1-2):22-33.
5. The New Gold Standard for Gene Therapy Protein Expression Potency Measurements. Bio-Techne Application Note. Link.
6. Giles AR, et al. Deamidation of Amino Acids on the Surface of Adeno-Associated Virus Capsids Leads to Charge Heterogeneity and Altered Vector Function. Mol Ther. 2018 Dec 5;26(12):2848-2862.
7. Zhou Y, Wang Y. Direct deamidation analysis of intact adeno-associated virus serotype 9 capsid proteins using reversed-phase liquid chromatography. Anal Biochem. 2023 May 1;668:115099.
8. Aronson SJ, et al. A Quantitative In Vitro Potency Assay for Adeno-Associated Virus Vectors Encoding for the UGT1A1 Transgene. Mol Ther Methods Clin Dev. 2020 Jun 3;18:250-258.
9. Martinez-Fernandez de la Camara C, McClements ME, MacLaren RE. Accurate Quantification of AAV Vector Genomes by Quantitative PCR. Genes (Basel). 2021 Apr 19;12(4):601.
10. Meierrieks F, et al. Unveiling the secrets of adeno-associated virus: novel high-throughput approaches for the quantification of multiple serotypes. Mol Ther Methods Clin Dev. 2023 Sep 20;31:101118.

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