



Abstract

ABL is a Rockville-based CDMO with over 63 years of industry experience in providing solutions for the development & manufacturing of oncolytic, cell and gene therapy and vaccine treatments. This study reveals the versatility of ABL's long-standing p24 ELISA kit (ABL® kit), originally designed for HIV titer determination, now proven to accurately detect lentivirus (LVV) titer of in-process samples during industrial lentiviral product purification. Comparative analysis against kits from four (4) other vendors reveals the ABL® kit to be superior, particularly in accurately quantifying low titer & early stage LVV purification samples, thus highlighting the ABL® kit as an accurate and reliable tool for LVV purification and titer determination in cell & gene therapy applications.

Introduction

ABL® kit: ABL's Legacy and Innovation. With over 40 years of experience, ABL has an extensive track record in human immuno-deficiency virus (HIV) research. The gag p24 is a reliable marker for HIV, and p24 ELISA kits are commonly used to determine HIV titers. ABL pioneered one of the first p24 ELISA kits in the market. Unlike some methods using recombinant techniques, all components in the ABL® kit ([Catalog #5421](#) & [#5447](#)) are derived from natural sources (Fig. 1).

Introduction- continued, see Fig. 1

- ABL p24 standard: purified from a cell line constitutively producing HIV-1.
- Capture antibodies: two (2) carefully selected monoclonal antibodies derived from mouse immunized with viral p24 protein.
- Detection antibody: polyclonal antibody purified from HIV-positive human plasma.

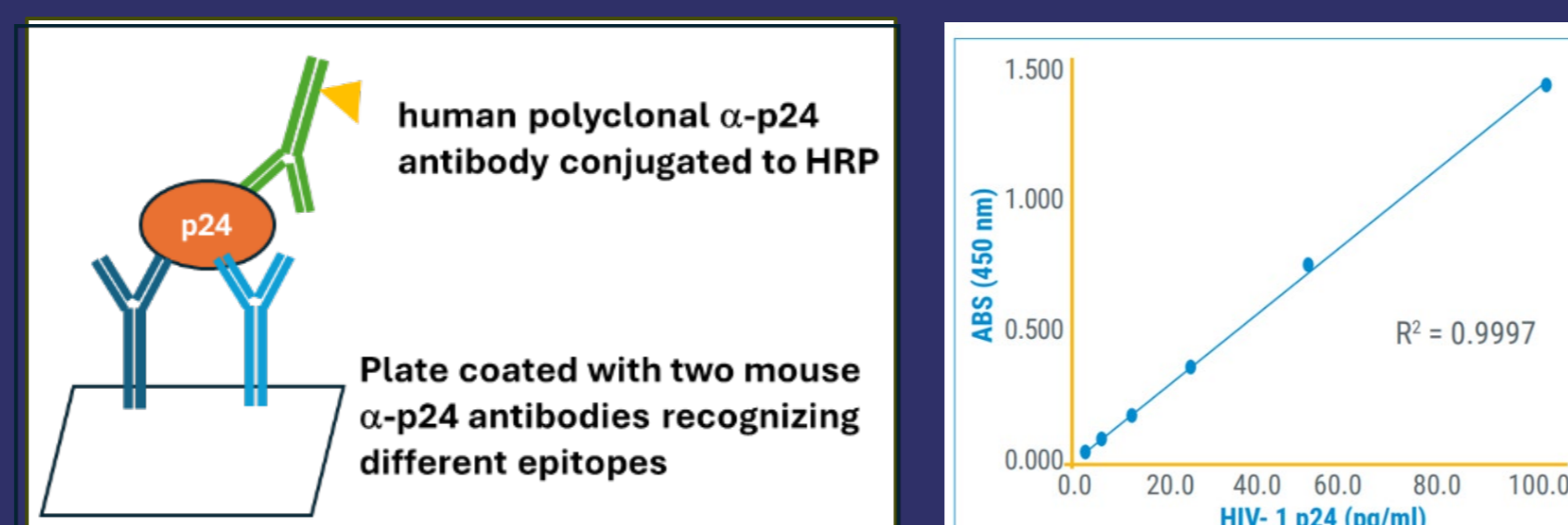


Figure 1. Left: key components of ABL's HIV p24 sandwich ELISA kit. Right: Example standard curve.

Features and benefits of the ABL® kit.

Sensitivity

- Quantitation down to picogram level: 3.1 to 100 pg/mL

Convenient

- Single plate and bulk formats
- Pre-formulated reagents
- Just 3 incubation steps
- Removable 8-well strips

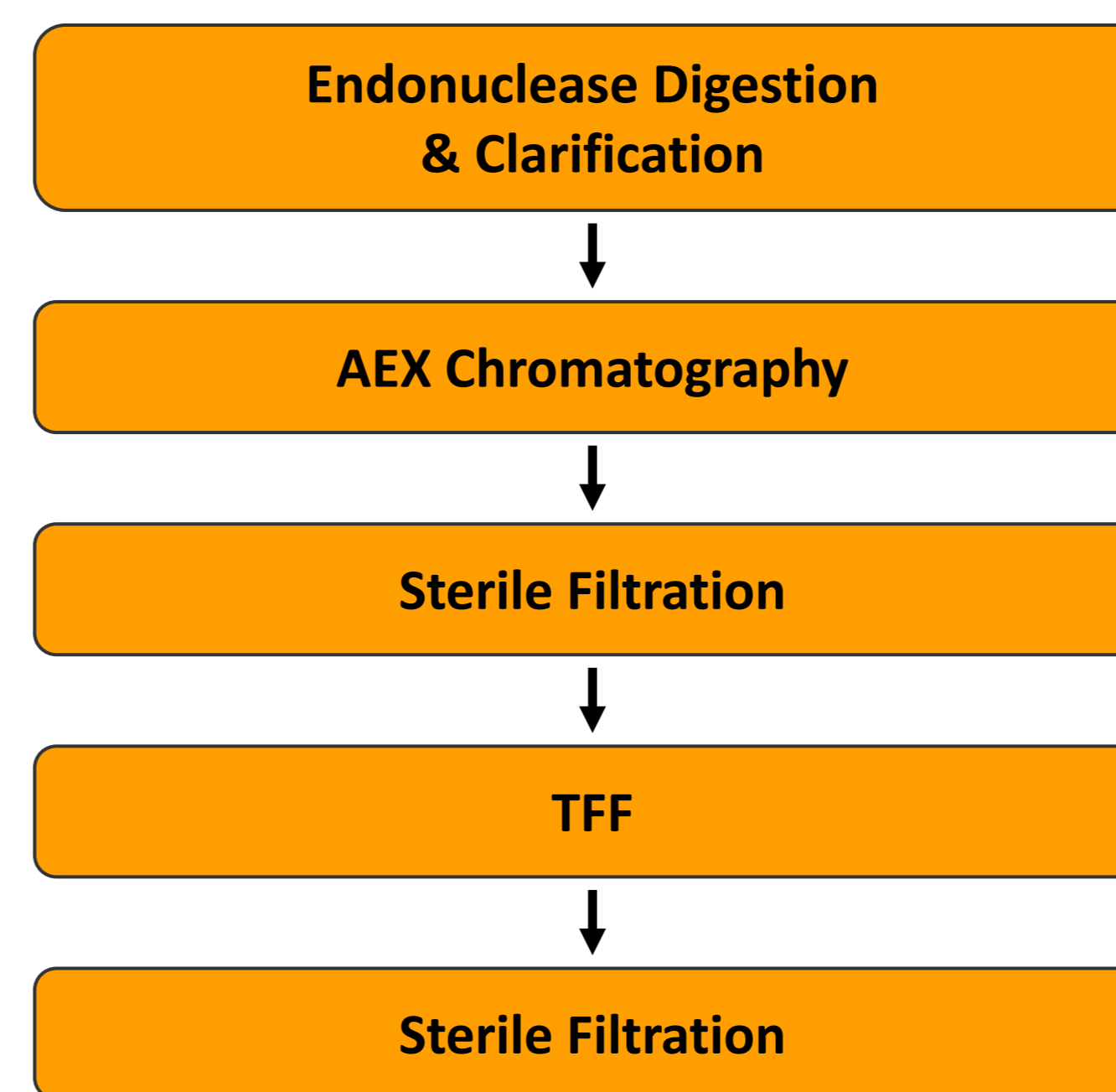
Efficient

- Low sample volume: 100 µL
- Results in < 3 hours

Introduction- continued, see Fig 2

ABL's Lentivirus (LVV) Purification Process. (Fig.2).

Figure 2. ABL LVV downstream purification process from 20 Liter production.



Challenges in quantifying in-process samples for LVV industrial production.

There is a poor correlation between genomic titer and p24 results when evaluating in-process LVV samples, most notably in early-stage and low-concentration samples. However, the ABL® kit exhibited remarkable reliability with such samples. Consequently, a comparison was performed to study the ABL® kit against those offered by other vendors.

Method

Each LVV in-process sample was aliquoted into multiple smaller volumes and tested via the ABL® kit against similar ELISA kits from four (4) other vendors: B, C, D & E. Genomic titer was measured by qRT-PCR. Two of the kits were selected for re-testing of all samples using different dilutions, ensuring consistent & reproducible results, characterized by CV <20%.

Samples were categorized into the following subgroups for analysis:

- Total Samples
- Lower Titer: GC Titer < 4.0E+09 vg/mL
- Higher Titer: GC Titer ≥ 4.0E+09 vg/mL
- Early Steps: Harvest, AEX Load.
- Later Steps: AEX eluate, TFF retentate & Sterile filtrate.

Correlation between virus particle (VP) from genomic titer and VP from p24 in every subgroup was evaluated using two methods:

- 1) R² (Coefficient of determination)
- 2) The average ratio of p24/GC, along with correlation co-efficiency (CV%).

Acronyms

vg: virus genomic copy #, measured by qRT-PCR.

VP: Virus Particles.

VP conc. (from p24) = p24 conc. (ng/mL) x 1.0E+07.

VP conc. (from GC) = GC conc. (vg/mL) ÷ 2.



Results

1. Cost effective & convenient protocol.

Compared to other commercial p24 kits, ABL's p24 ELISA kit offers a cost advantage, with an incubation time of 2.5 hours and a three-step incubation process (Table 1).

	ABL	Kit-B	Kit-C	Kit-D	Kit-E
Price	\$	\$	\$\$\$	\$\$\$	\$\$
Operation Time (hrs)	2-3.5	2-3.5	< 2	> 3.5	> 3.5
# of Steps	3	4	2	4	3

\$: 450-600;
 \$\$: 600-750;
 \$\$\$: 750-900

Table 1. Cost & operation comparisons.

2. Correlation calculated by R².

Testing LVV in-process samples with the ABL® kit shows strong correlation (> 0.92 R² in all subcategories) with genomic titer, especially in low titer and early step samples (Table 2 & Fig. 3).

Correlation of p24 with GC (R ² value)					
	ABL	Kit-B	Kit-C	Kit-D	Kit-E
All Samples	0.984	0.976	0.839	0.981	0.989
Low Titer	0.919	0.890	0.712	0.877	0.887
High Titer	0.981	0.971	0.825	0.980	0.990
Early Step	0.961	0.816	0.836	0.845	0.852
Late Step	0.992	0.981	0.990	0.988	0.998

Table 2. R² values indicating correlations of GC with p24 for each kit and in each sample subcategory. The darker green indicates stronger correlation among each sub-group.

Notes

- ✓ ABL's p24 ELISA Kit is for research use only
- ✓ ABL is converting the assay to support cGMP

Results

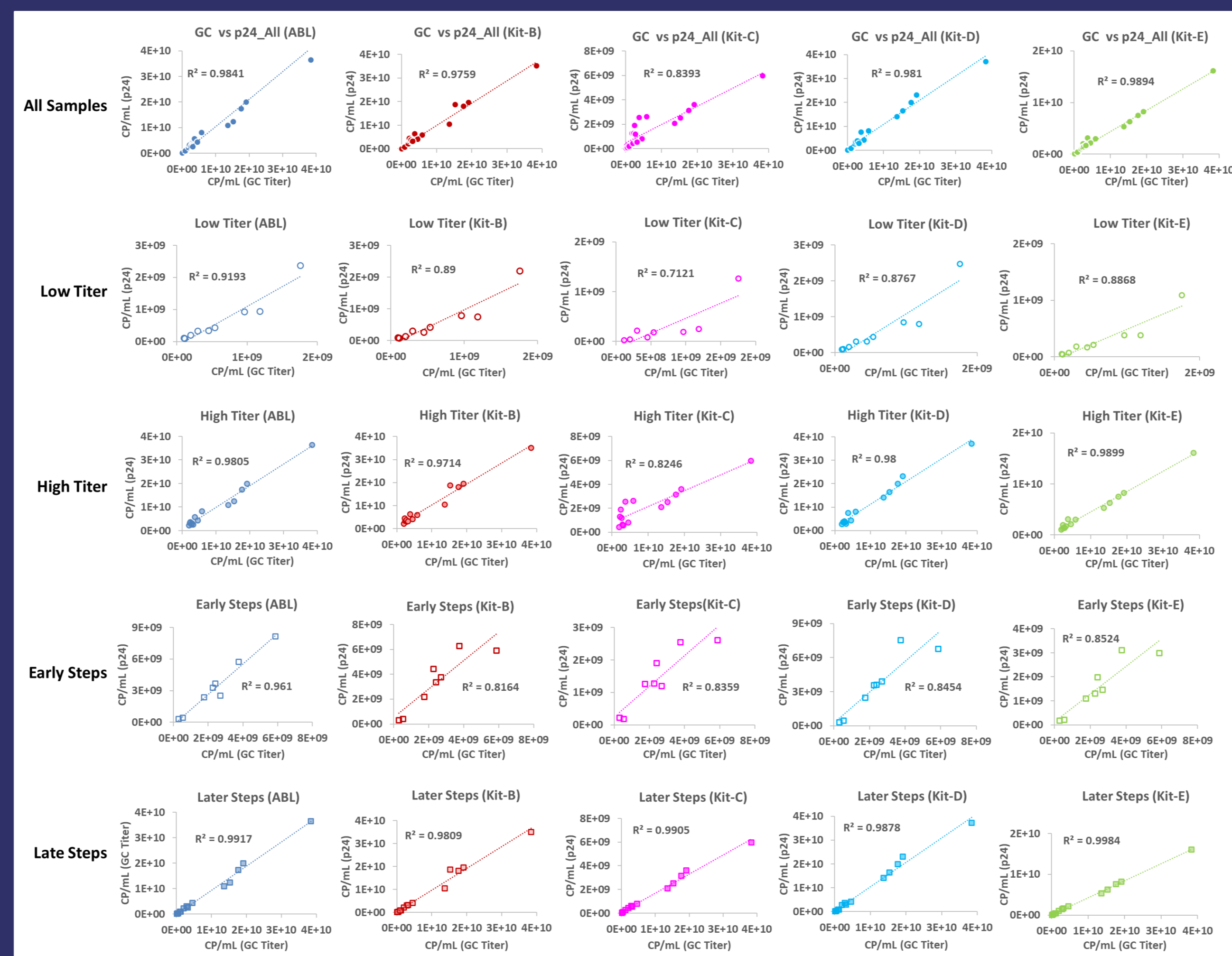


Figure 3. Graphs show p24-derived Virus particle concentration (VP/mL) plotted against genomic copies (GC)-derived VP concentrations using linear regression. The trendline and correlation (R²) factor are shown on each graph.

Ordering Information for the ABL® kit

Single kit (1 x 96 Well Plate, Cat# 5421)

<https://ablinc.com/product/5421-hiv-1-p24-antigen-capture-assay/>

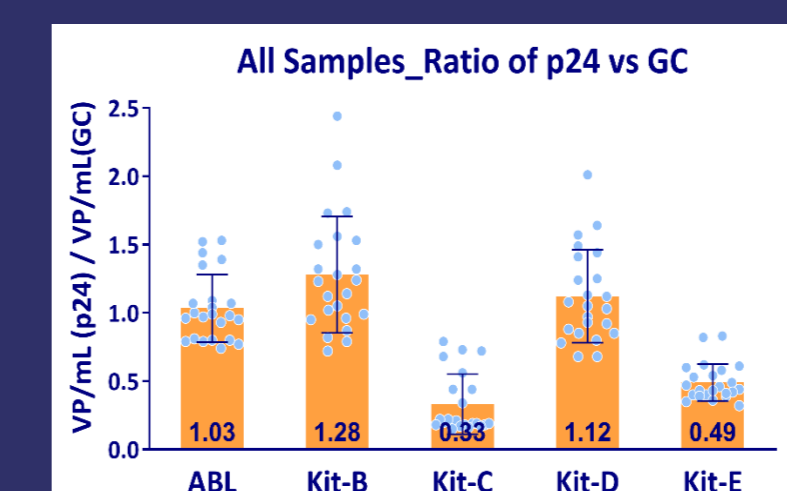
Bulk order (10 x 96 Well Plate, Cat# 5447)

<https://ablinc.com/product/5447-bulk-hiv-1-p24-10-plateskit>

Results

3. p24/GC ratio & CV%.

Higher titer samples have more impact on R² than lower titer samples. Thus, ABL used an alternative approach by calculating the average p24/GC ratio and employing CV% to gauge correlation strength within subgroups. The average ratio for LVV in-process samples with the ABL kit is 1.03, consistent with the equivalence of 1 ng p24 = 1.0E+07 VP. Notably, it exhibited the lowest CV%, indicating robust correlation, especially with lower titer and early-step samples (Fig.4).



	Average					CV%				
	ABL	Kit-B	Kit-C	Kit-D	Kit-E	ABL	Kit-B	Kit-C	Kit-D	Kit-E
Total	1.03	1.28	0.83	1.12	0.49	24%	33%	67%	30%	27%
Lower Titer	0.94	0.99	0.34	0.89	0.43	21%	26%	70%	26%	25%
Higher Titer	1.09	1.46	0.32	1.27	0.53	24%	28%	67%	24%	26%
Early Step	1.25	1.63	1.88	1.37	0.61	23%	30%	28%	27%	25%
Late Step	0.92	1.09	0.59	0.97	0.43	12%	23%	7%	20%	6%
VP / ng p24	1.00E+07	1.25E+07	1.00E+07	1.00E+07	1.00E+07					

Figure 4. The average ratios of viral particle concentration (VP/mL) calculated from p24 over GC for each subgroup. **Left:** A bar graph displaying average ratios and sample distributions of all the kits in "All sample" subcategory. **Right:** The table lists average ratios and CV% for all kits across all subgroups. Darker green in the CV% columns indicate stronger correlation within each subgroup.

Conclusions

ABL's HIV p24 ELISA kit: TOP CHOICE for quantifying Lentivirus Vector in-process superior samples.

- ☐ Cost-effective, moderate incubation time & reasonable number of operation steps
- ☐ Satisfactory R² values of p24 with GC in all LVV in-process samples, especially in low-titer & early-step samples.
- ☐ Favorable p24/GC ratios with less variations among LVV in-process samples.