

# ABL's HIV p24 ELISA Kit: a Solution for Lentivirus Quantification in Industrial Processes

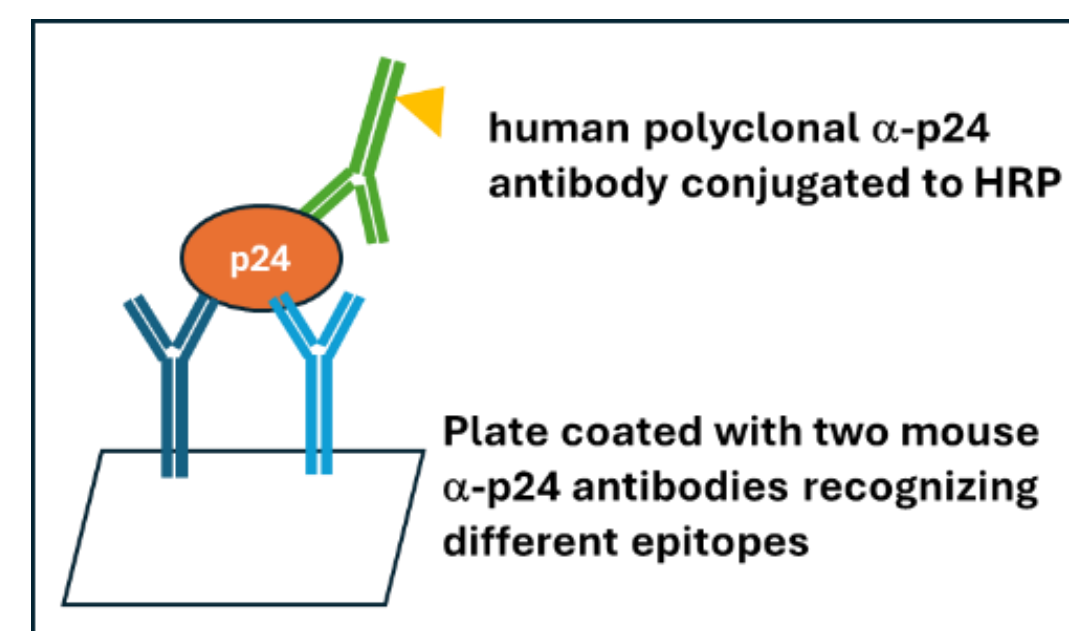
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Abstract

Since the discovery of HIV-1 in the 1980s, ABL has been a pioneer in HIV research, including the development of one of the first p24 ELISA kits (ABL® kit, Catalog #5421 & #5447) for the reliable quantification of HIV capsid protein (p24). Today, ABL provides manufacturing and analytical services for oncolytic viruses, cell and gene therapies, and vaccines. Recently, ABL developed a platform for the industrial production and purification of lentiviral vectors (LVV). As LVV share structural and biological similarities with HIV, we evaluated the ability of ABL's HIV p24 kit to quantify LVV in in-

process samples generated during industrial production and purification. One challenge in quantifying such samples is the poor correlation between genomic titer (viral particle titer derived from RNA content) and p24 results (viral particle titer derived from LVV capsid marker (p24)), particularly in early-stage and low-concentration samples. However, the ABL® kit exhibited remarkable reliability when analyzing such samples. To further explore this observation, we compared the performance of the ABL® kit to those offered by other vendors.

## Introduction: ABL® kit- ABL's Legacy for HIV Titer Determination

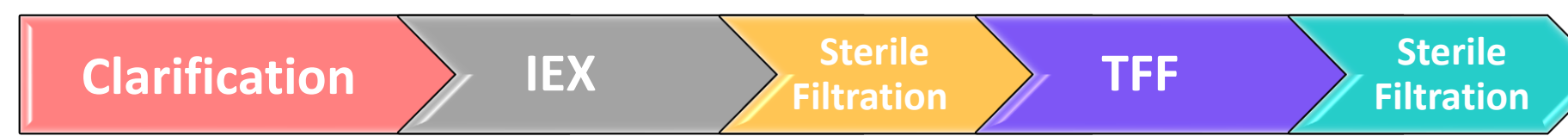


Unlike some methods using recombinant materials, all components in the ABL® kit are derived from natural sources.

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

## Introduction: ABL's Lentivirus Purification Process and Industrial Production Challenge

ABL's downstream Lentivirus purification scheme (20 L)



**Challenges in the field:** 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 four other vendors.**

## Method

LVV in-process samples were tested in replicate at two dilutions using the ABL® kit as compared to p24 ELISA kits from four (4) other vendors: B, C, D & E (ng/mL). Genomic titers (vg/mL) were measured by RNA extraction & qRT-PCR.

**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 ret. & Sterile filtrate

**Virus particle conc. (VP/mL) were calculated as follows:**

- **VP-p24** (VP/mL from p24) = p24 conc.(ng/mL) x 1.0E+07
- **VP-GC** (VP/mL from genomic titer) = virus genomic copy conc. (vg/mL) ÷ 2.

**Correlation Analysis** between vector genome and p24 ELISA-based particle concentration was evaluated across all subgroups using two methods:

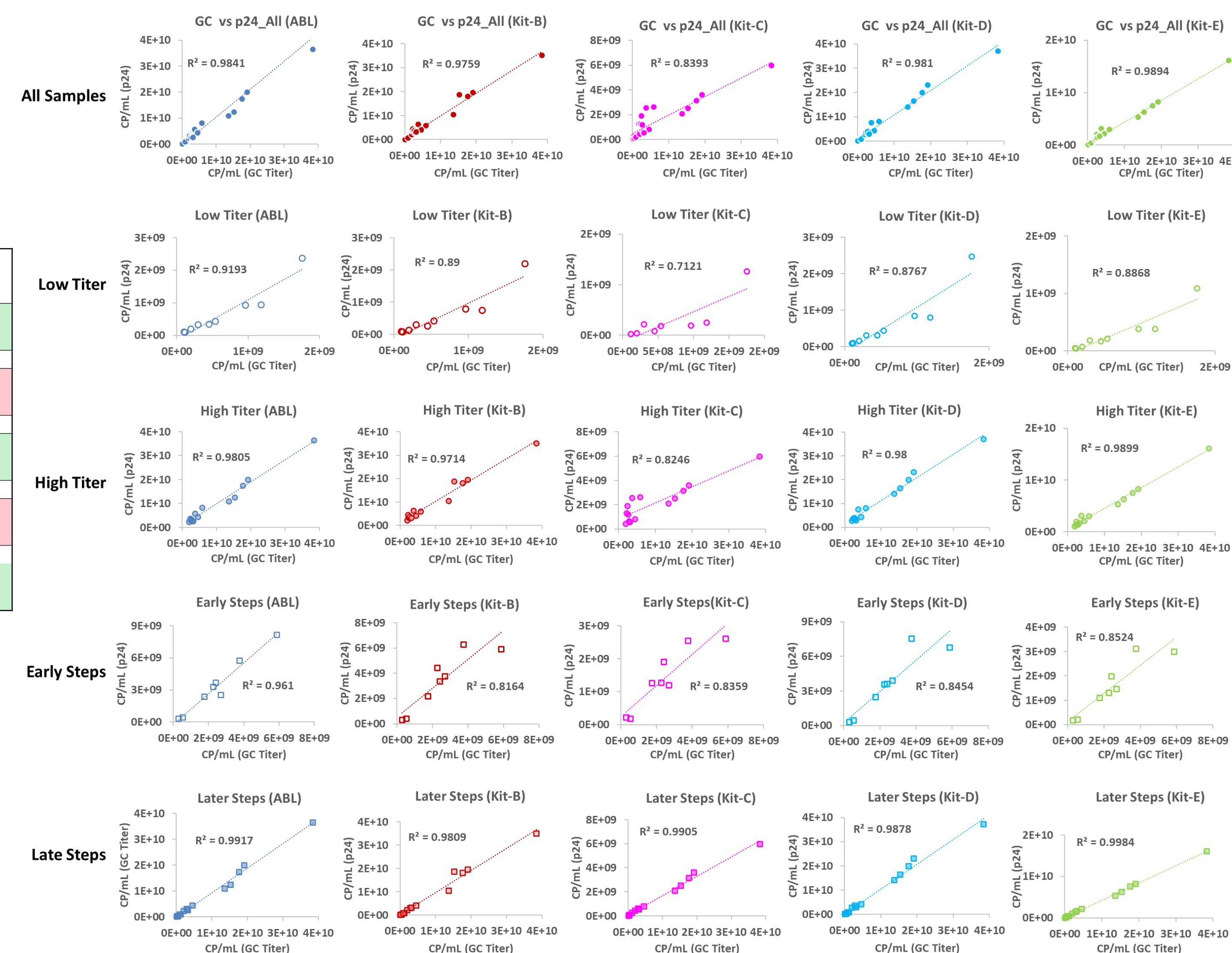
1. **R<sup>2</sup>** (Coefficient of determination)
2. **Average ratio & CV% of VP-p24/VP-GC Ratio** (R<sup>2</sup> is influenced more heavily by high-titer samples)

## Results: Correlation calculated by R<sup>2</sup>

Correlation of p24 with GC (R<sup>2</sup> value)

	ABL	Kit-B	Kit-C	Kit-D	Kit-E
<b>All Samples</b>	0.984	0.976	0.839	0.981	0.989
<b>Low Titer</b>	0.919	0.890	0.712	0.877	0.887
<b>High Titer</b>	0.981	0.971	0.825	0.980	0.990
<b>Early Step</b>	0.961	0.816	0.836	0.845	0.852
<b>Later Step</b>	0.992	0.981	0.990	0.988	0.998

As compared to all other kits, testing LVV in-process samples with the ABL® kit shows strong correlation (> 0.92 R<sup>2</sup> in all sub-categories) with genomic titer, especially in low titer and early step samples.

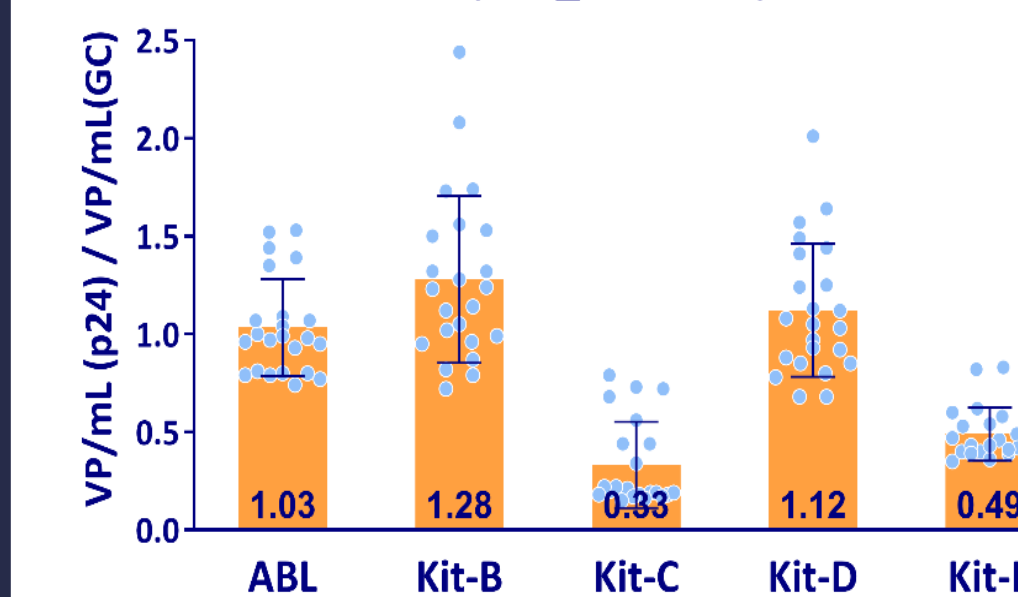


## Results: Average and CV% of VP-p24/VP-GC ratio

Ratios: VP-p24 / VP-GC

	Average					CV%				
	ABL	Kit-B	Kit-C	Kit-D	Kit-E	ABL	Kit-B	Kit-C	Kit-D	Kit-E
<b>Total</b>	1.03	1.28	0.33	1.12	0.49	24%	33%	67%	30%	27%
<b>Lower Titer</b>	0.94	0.99	0.34	0.89	0.43	21%	26%	70%	26%	25%
<b>Higher Titer</b>	1.09	1.46	0.32	1.27	0.53	24%	28%	67%	24%	26%
<b>Early Step</b>	1.25	1.63	1.88	1.37	0.61	23%	30%	28%	27%	25%
<b>Later Step</b>	0.92	1.09	0.59	0.97	0.43	12%	23%	6.7%	20%	6.1%
<b>VP/ng p24</b>	1.0E+07	1.25E+07	1.0E+07	1.0E+07	1.0E+07					

All Samples\_Ratio of p24 vs GC



- Higher titer samples have more impact on R<sup>2</sup>. Thus, ABL used an alternative approach: calculating the average p24/GC ratio and employing CV% to gauge correlation strength within subgroups.
- Compared with all the other kits tested, ABL Kit exhibited the lowest CV%, indicating robust correlation, especially with lower titer and early-step samples.
- 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.

## Cost effective & Convenient Protocol

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

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

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

## Notes

- ABL® p24 ELISA Kit is for research use only
- ABL is converting the assay to support cGMP LVV production.

Summary

**ABL's HIV p24 ELISA kit: A TOP CHOICE for quantifying Lentivirus Vectors in in-process samples among all p24 kits.**

- Strong R<sup>2</sup> values when comparing 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 compared to all other four kits analyzed.
- Cost-effective, with moderate incubation time & reasonable number of operation steps.

**The p24 ELISA test offers several advantages over the GC titer test:**

- ◆ Greater simplicity ◆ Faster turnaround ◆ Lower variability
- ◆ Reduced sample volume requirements ◆ Less stringent demands on sample quality



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