FASTFACTS

Analyzing lentivirus particles using dPCR techniques

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Lentiviral vectors (LVVs) are an active ingredient in biotherapeutics and must be tested for identity, purity, potency, safety, and stability according to regulatory guidelines. Therefore, reliable methods to characterize and quantify LVVs are critical to the success of many cell and gene therapies. This FastFacts poster explores an innovative digital PCR (dPCR) technology; a method for absolute quantification of nucleic acids, without standard curves.

WHAT IS dPCR?

dPCR is a method of quantifying nucleic acid targets by dividing the bulk PCR reaction into thousands of smaller, independent reactions. This method does not require a standard curve and is capable of providing absolute quantification of known genetic targets. Absolute quantification of a sample is achieved by counting positive reactions and applying Poisson statistics. Because no standard curve is required, this method is considered to offer greater precision and reproducibility when compared to other quantitative methods, even in high-background conditions.

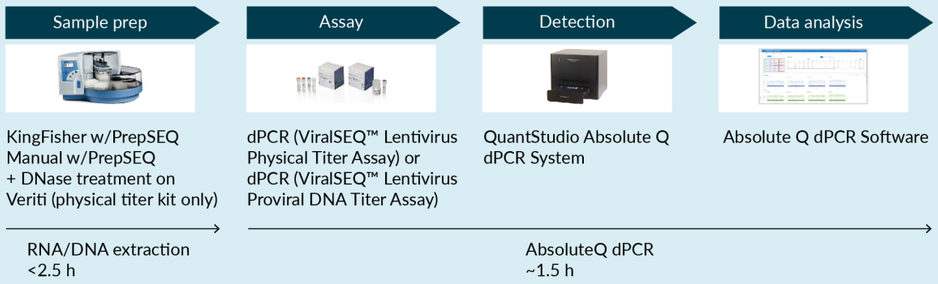
Limitations of existing PCR platforms include: significant wastage of sample; limited or inconsistent compartmentalization; tedious workflow (with multiple instruments and extensive human intervention required); long turnaround times (6+ h to generate a single data point); and the limited insight derived from endpoint analysis alone (leading to greater potential for a false positive result).

FLEXIBLE, INTEGRATED SOLUTION FROM PROCESS DEVELOPMENT TO GMP

The Applied Biosystems™ QuantStudio™ Absolute Q™ dPCR System addresses these limitations by providing:

- Reagent efficiency—<5% wasted reagent
- Consistency—20,000 consistent microreactions per array
- Easy-to-use workflow, equivalent to a qPCR system
- Fast time-to-results—90-minute run-time
- Confidence in data—automatic false positive rejection
- A single instrument
- Flexibility—4–16 samples per run
- Multiplexing—4-color capacity

Figure 1. EMA recommended standards.

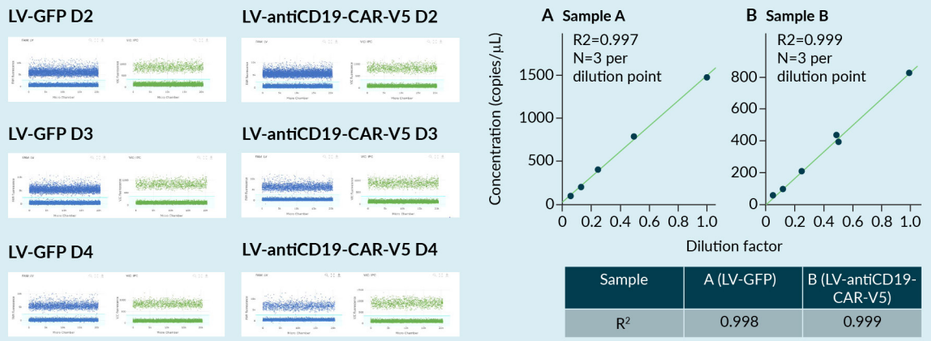


Two titer kits for quantitiation of LVVs can be used with the Absolute Q dPCR System: the ViralSEQ™ Lentivirus Physical Titer Assay and the ViralSEQ Lentivirus Proviral DNA Titer assay. These kits allow for the end-to-end workflow solution shown in **Figure 1**.

PHYSICAL TITER ANALYSIS

The analysis obtained for physical titer using the Absolute Q dPCR System is presented in **Figure 2**. The data on the left shows the fluorescence intensity (y-axis) of all microchambers (x-axis) for a given sample.

Figure 2. 'GMP-Like' versus GMP-Now™ plasmid DNA.



Sample	A (LV-GFP)	B (LV-antiCD19-CAR-V5)
R^2	0.998	0.999

Microchambers positive for the target on the lentiviral vector were FAM dye-labeled (shown in blue plot) and are above the blue threshold line; microchambers for the VIC dye-labeled internal positive control (shown in green plot) are also shown to be above the threshold. The data on the right illustrates the linearity of the dilution for the two samples, (A) LV-GFP and (B) LV-antiCD19-CAR-V5.

Figure 3 highlights the concentrations determined by the Absolute Q dPCR System and the adjusted concentrations in copies/mL and viral particles/mL. After accounting for the serial dilution factors, the concentrations were consistent, reflecting the high precision and accuracy of the assay. The total dilution factor was calculated using equation 1, then leveraged in equations 2 and 3 using the average dPCR concentration to determine the lentiviral stock concentration and the physical titer.

For research use only. Not for use in diagnostic procedures.

Figure 3. Calculating physical titer.

1 ▶ Total dilution factor=extraction dilution × DNase dilution × serial dilution × reaction dilution (e.g., for sample A, dilution D2 → total dilution factor=2 × 2 × 1250 × 5=2.5 × 10^4)

2 ▶ LV stock in copies/mL=average dPCR read × total dilution factor × 1000 µL/mL

3 ▶ LV physical titer in VP/mL=LV stock ÷ 2

LV Sample	Dilution	Average dPCR read copies/µL)	CV of replicates	Total dilution factor	LV stock (copies/mL)	LV physical titer (VP/mL)
A (LV-GFP)	D2	1479.29	0.97%	2.5×10^4	3.70×10^{10}	1.85×10^{10}
	D3	786.21	0.97%	5.0×10^4	3.93×10^{10}	1.97×10^{10}
	D4	395.99	1.60%	1.0×10^5	3.96×10^{10}	1.98×10^{10}
	D5	196.73	2.05%	2.0×10^5	3.93×10^{10}	1.97×10^{10}
	D6	96.57	1.76%	4.0×10^5	3.86×10^{10}	1.93×10^{10}
B (LV-anti CD19 –CAR-v5)	D2	822.21	0.43%	2.5×10^4	2.06×10^{10}	1.03×10^{10}
	D3	403.06	2.91%	5.0×10^4	2.02×10^{10}	1.01×10^{10}
	D4	202.61	3.33%	1.0×10^5	2.03×10^{10}	1.01×10^{10}
	D5	97.50	3.91%	2.0×10^5	1.95×10^9	9.75×10^{10}
	D6	50.56	1.82%	4.0×10^5	2.02×10^{10}	1.01×10^{10}
Positive control	NA	1368.66	4.47%	NA	NA	NA
Negative control	NA	0	NA	NA	NA	NA
No-template control	NA	0	NA	NA	NA	NA

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