Simplifying Progress

Rapid, Real Time Quantification of Lentivirus Particles Using Antibody-Based Detection on the Virus Counter® 3100 Platform

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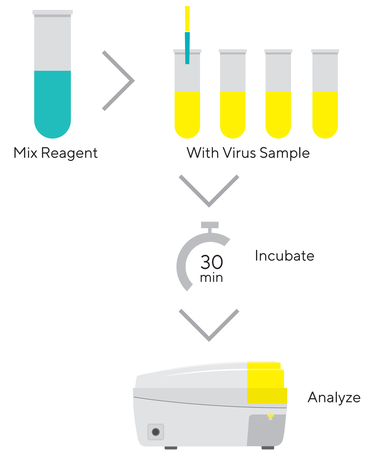


Abstract

Lentivirus particles are valuable vectors for modern gene and cell therapies. Due to severe setbacks in early gene therapy clinical trials, accurate enumeration of total particle count of gene therapy vectors is critical in order to minimize the risk of adverse immune response or other negative outcomes when using viral vectors.

Quantification of Lentivirus particles is challenging, often relying upon difficult and variable methods such as ELISA and qRT-PCR. Rapid and precise analytical methods are needed to monitor vector production and enumerate particles in final formulations. The Virus Counter® 3100 instrument and antibody-based Virotag® VSVG reagent allow for the rapid quantitation of Lentivirus particles by utilizing serotype-specific fluorescently labeled antibodies with high affinity to intact Lentivirus particles expressing the VSV-G epitope.

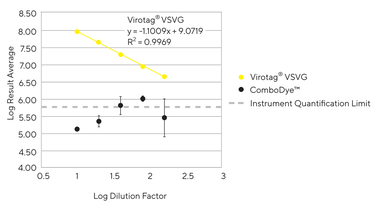
In conjunction with the ComboDye™ reagent, comparisons of epitope expression and capsid integrity can reveal more information necessary for process optimization. Other rapid viral quantification methods that quantify total genome copy (qRT-PCR) and viral antigen (ELISA) concentration, respectively, may quantify unassociated nucleic acid and unassembled viral antigens, leading to inaccurate estimates of Lentivirus particle concentrations.

Here we demonstrate that the Virus Counter® 3100 instrument coupled with the Virotag® VSVG reagent represents a rapid, biologically-relevant method of quantification for Lentivirus samples and BacMam particles expressing VSV-G pseudotype, while specifically excluding non-VSV-G expressing Baculovirus and other negative controls. Utilizing a patented, no-wash assay, Lentivirus samples are stained in 30 minutes and then counted in 3 minutes per sample. This speed allows for in-process monitoring and production optimization of Lentivirus vector products, making the Virus Counter® 3100 instrument and Virotag® reagents a valuable addition to bioprocessing applications utilizing Lentivirus particles.

Results

A

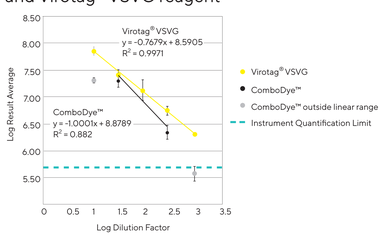
Crude Lentivirus, ComboDye™ reagent and Virotag® VSVG reagent



Triplicate measurements within a dilution series of a crude Lentivirus preparation demonstrates high precision as well as high linearity using the Virotag® reagent; whereas, ComboDye™ stain failed to generate a detectable signal due to interaction with interfering material in the sample.

B

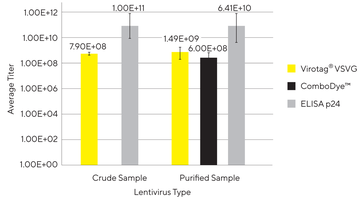
Purified Lentivirus, ComboDye™ reagent and Virotag® VSVG reagent



The novel VSVG antibody stain demonstrates enhanced precision and linearity for the entire dilution range of samples; whereas, the dynamic range of the assay was limited using the ComboDye™ reagent where interactions with other material in the virus preparation led to premature saturation of signal and an inability to detect virus in highly diluted samples.

C

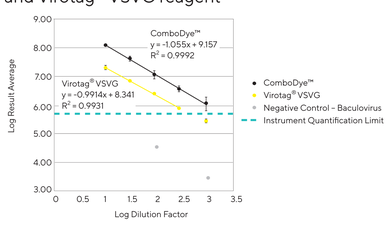
Comparison of Quantification Methods



Viral titer was calculated by multiplying raw instrument counts by associated dilution factors. A comparison of titers across ComboDye™ reagent, Virotag® VSVG reagent, and ELISA p24 assays demonstrated that quantification via ELISA was up to 2 orders of magnitude higher than Virotag® VSVG analysis. The antibody stain also demonstrated higher particle counts than the ComboDye™ stain. ComboDye™ stain failed to detect virus in the commercial sample (presumably due to interfering material in the medium) as seen in Figure A.

D

Crude BacMam, ComboDye™ reagent and Virotag® VSVG reagent



BacMam constructs displaying VSV-G proteins can also be quantified linearly and precisely by the Virotag® VSVG. Baculovirus is not recognized by the same reagent due to the lack of VSV-G expression. Additional negative controls consisting of Adenovirus 5, Influenza | B | Phuket | 3073 | 2013, and blanks, also fail to demonstrate significant antibody binding. BacMam samples stained using ComboDye™ reagent demonstrate slightly higher counts due to the lower specificity of the dual stain approach.

Discussion & Conclusion

Traditional ELISA and qPCR methods can be time-consuming and highly variable, while also yielding incomplete data. Methods to discern infective from non-infective virions in addition to those that express demonstrable p24 and genomic content are needed to support the understanding of total viral count. As emerging state-of-the art technologies, ComboDye™ and Virotag® VSVG methods provide unique insight into the levels of non-infective particles in virus preparations.

- The use of VSVG antibody stain with the Virus Counter® instrument provides more precise and accurate quantification of total particles.
- The use of VSVG antibody stain with the Virus Counter® instrument improves specificity to allow previously difficult early process samples to be quantified.
- The unique specificity of the Virotag® VSVG reagent demonstrates such high specificity that VSV-G-expressing BacMam particles can be detected while excluding native Baculovirus and other negative controls.
- Crude Lentivirus samples, that were previously difficult or impossible to quantify due to complex matrices, can now be assessed, resulting in decreased noise (improved linearity) and increased precision.
- The VSVG antibody stain avoids non-specific binding and thus extends the dynamic range of the assay by improving signal: noise ratios.
- Virotag® VSVG reagent demonstrated reduced standard errors compared to ELISA assays and the ComboDye™ stain on the Virus Counter® platform.
- The Virotag® VSVG reagent demonstrated lower counts in comparison with the ELISA method, suggesting overestimation of total particle counts by the ELISA approach, likely due to recognition of unassociated proteins and virus fragments in the assay.