SARTORIUS

Application Note

December 03, 2018

Keywords or phrases:
Viral Vector, Lentivirus, Total Particle Quantification, Gene Therapy, Cell Therapy, Virus Counter® Platform

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

Tyler Gates, Rebecca Montange PhD, Antje Schickert PhD, Katherine D. Shives PhD, Jeff Steaffens MS
Sartorius Stedim North America Inc., 6542 Fig St., Arvada, CO 80004, 720 599 3700

Correspondence
Email: Antje.Schickert@Sartorius.com

Abstract

Lentivirus particles are valuable vectors for modern gene and cell therapies. Severe setbacks in early clinical trials have shown that 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.

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 VSV-G pseudotyped BacMam particles, 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.

Introduction

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 platform is a technology that enables direct virus quantification. To date, the Combo Dye® reagent has been used to label viral particles: this technology continues to provide broad applicability for the counting of a number of enveloped viruses in the absence of more specific labels like antibodies conjugated with fluorescent molecules. In an effort to expand the scope of the Virus Counter® platform to non-enveloped viruses and to address the need for reagents that will function with crude samples, we developed a number of antibody reagents for use on the Virus Counter® instrument. Other rapid viral quantification methods that quantify total genome copy (qRT-PCR) and viral antigen (ELISA) concentration, may quantify unassociated nucleic acid and unassembled viral antigens respectively, leading to inaccurate estimates of Lentivirus particle concentrations. The Virus Counter® 3100 instrument and antibody-based Virotag® VSVG reagent allow for the direct, rapid and precise quantitation of total Lentivirus particles by utilizing serotype-specific fluorescently labeled antibodies with high affinity for intact Lentivirus particles expressing the VSV-G epitope.

Results

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, Combo Dye® and Virotag® VSVG methods provide unique insight into the levels of non-infective particles in virus preparations.

- The Virus Counter® instrument together with the Virotag® VSVG reagent provide more precise quantification of total particles.
- The high specificity of the antibody-based Virotag® VSVG stain allows previously difficult, early process samples to be quantified.
- The unique specificity of the Virotag® VSVG reagent ensures that VSV-G expressing BacMam particles can be detected while excluding native Baculovirus and other negative controls.
- The Virotag® VSVG reagent avoids non-specific binding and thus extends the dynamic range of the assay by improving signal:noise ratios.
- Virotag® VSVG reagent has reduced standard errors compared to ELISA assays.
- The Virotag® VSVG reagent demonstrates 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.

Crude Lentivirus, Virotag® VSVG Reagent

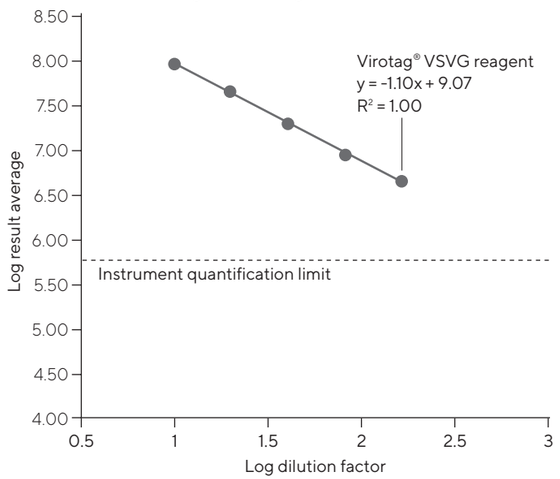

Figure 1: Triplicate measurements within a dilution series of a crude Lentivirus preparation demonstrates high precision as well as high linearity using the Virotag® VSVG reagent.

Purified Lentivirus, Combo Dye® Reagent and Virotag® VSVG Reagent

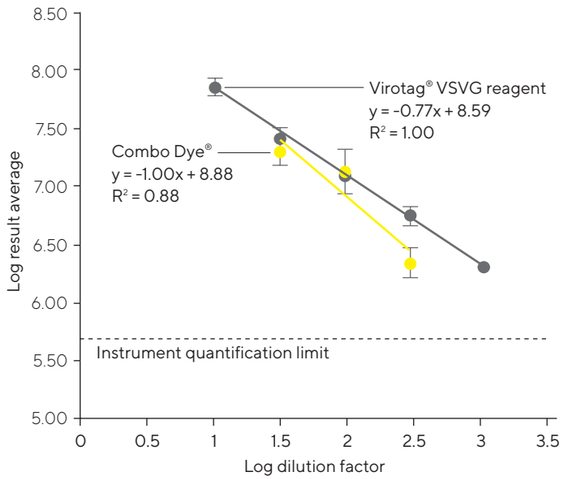

Figure 2: Novel Virotag® VSVG reagent demonstrates enhanced precision and linearity for the entire dilution range of samples.

Comparison of Quantification Methods

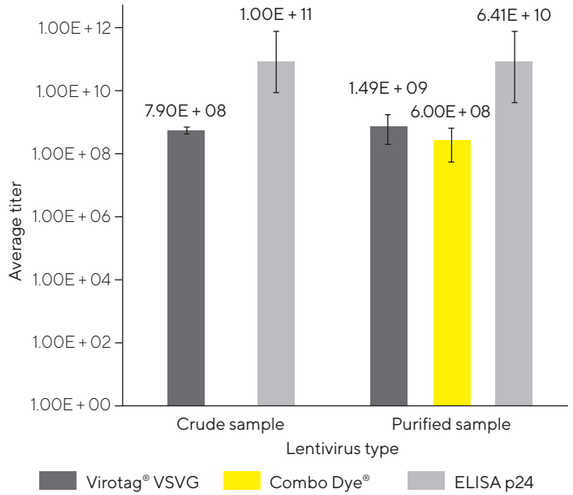

Figure 3: Comparison of titers across Combo Dye® reagent, Virotag® VSVG reagent, and ELISA p24 assays demonstrated that quantification via ELISA appeared to overestimate particle counts in the sample, likely due to recognition of unassociated proteins and virus fragments in the assay. The antibody stain also demonstrated considerably higher precision versus ELISA.

Crude BacMam and Virotag® VSVG Reagent

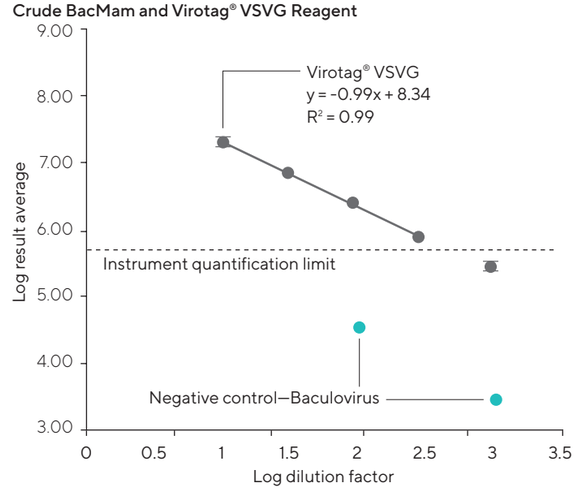

Figure 4: Baculovirus expressing VSV-G protein via the introduction of BacMam constructs are also measured linearly and precisely by the Virotag® VSVG reagent. As expected, native Baculovirus is not recognized using the same reagent. Additional negative controls consisting of Adenovirus 5, Influenza | B | Phuket | 3073 | 2013, and blanks, also fail to demonstrate significant antibody binding (data not shown).

Conclusion

As emerging state-of-the art technologies, the Virus Counter® 3100 instrument and Virotag® VSVG reagent provide rapid and direct quantification of total viral particles in virus preparations.

- Real-time titer insights, in-process monitoring and optimization of Lentivirus vector production
- A rapid, biologically relevant method of Lentivirus quantification
- Quantification utilizing fluorescently labeled antibodies with high affinity
- High specificity allows previously difficult early process samples to be quantified
- Reduced standard errors compared to ELISA assays

References

Artinger M, *et al.* **Virotherapy Process Optimization.** *Bioprocessing Journal,* 14(1) (2015)

Artinger M, *et al.* **Direct, Real-Time Antibody-Based Quantification of Baculovirus.** *BioProcessing Journal,* 14(4);15-21 (2016)

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For further information,
visit www.sartorius.com

Material-No.: 85037-561-84
Publication No.: SL-4088-e
Status: 11 | 2020