Technical note

Improved AAV Particle Protection with J.T.Baker® Cell Lysis Solution

Significant technological advancements have been made over the past several years in the treatment and control of life threatening diseases. One of the most revolutionary of these advances is cell and gene therapy. Adeno-associated virus (AAV) has emerged as a leading platform for gene delivery for treating various diseases due to its excellent safety profile and efficient transduction to various target tissues. However, the large-scale production and long-term storage of viral vectors is an inefficient process resulting in lower yields, moderate purity, and shorter shelf-life compared to recombinant protein therapeutics.

The manufacturing of AAV includes several upstream, downstream and fill/finish unit operations. One of the key downstream unit operations is cell lysis where outer boundary or cell membrane is broken down or destroyed to release viral vector from the host cell. During cell lysis and separation of the viral vector from cell debris, viral vectors are exposed to harsh shear stress that aggregates and precipitates the virus vectors. None of the current lysis methods are known to prevent viral vector loss due to shear stress during processing. Therefore, there is a clear need for an easy to use cell lysis method that is not only efficient in lysing the cells but also protects the viral vector from damage during downstream processing.

In this study, we evaluated the J.T.Baker® Cell Lysis Solution for efficiency in protecting viral vectors from shear stress induced damage during the manufacturing process, resulting in improved viral vector titer. This novel cell lysis solution is composed of non-ionic detergents and additives that are benign, environmentally friendly and biodegradable according to OECD 301.

Materials and Methods

To determine the effectiveness of J.T.Baker® Cell Lysis Solution to improve viral vector yield and AAV particle integrity, AAV2 control particles carrying green fluorescent protein (GFP) (Vigene, cat# RS-AAV2-FL) were resuspended in 2 mL PBS solution at 1.82x1010 vg/mL in a 5 mL glass bottle. 20 µL of Triton™ X-100 or J.T.Baker® Cell Lysis Solution was added to reach the same final detergent concentration. Both solution mixtures were then exposed to shear stress using a magnetic bar and stir plate (250 RPM, 1 hour) at ambient temperature, to mimic 'worst case' scenario manufacturing processing conditions. Post shear stress, viral vector titer was quantified via qPCR targeting the GFP transgene.

J.T.BAKER® CELL LYSIS SOLUTION PROTECTS AGAINST SHEAR STRESS DAMAGE

The change in AAV2 genome titer was compared for J.T.Baker® Cell Lysis Solution and Triton X-100 under induced shear stress conditions. Following 1 hour of stirring, AAV2 titer for both lysis solutions was measured. Figure 1 shows the change in viral titer for J.T.Baker® Cell Lysis Solution and Triton X-100 from the average of 10 measurements after shear stress. Viral particles treated with J.T.Baker® Cell Lysis Solution showed improved recovery under shear induced conditions, compared to those treated with Triton X-100, with approximately 15% higher titer. Although the study demonstrated up to 15% higher titer for J.T.Baker® Cell Lysis Solution, it may vary with AAV serotype and operating conditions.

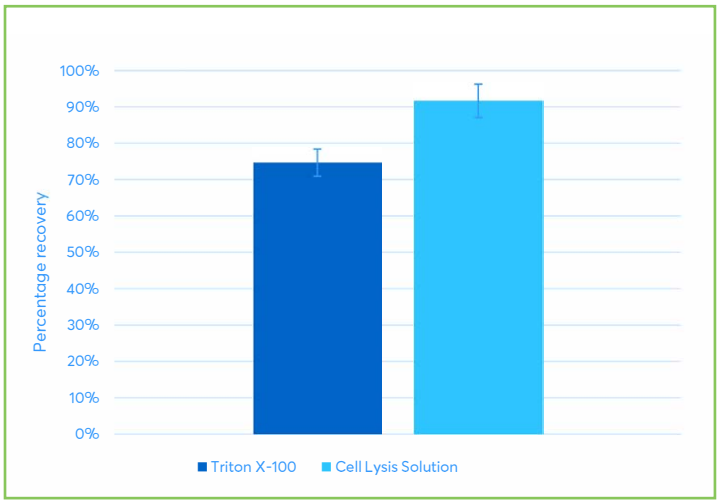

Figure 1: Recovery of AAV vectors following 1 hour agitation and purification, determined by qPCR titer.

CONCLUSIONS

J.T.Baker® Cell Lysis Solution demonstrated increased protection of AAV2 vectors from shear stress damage during the manufacturing process, resulting in improved vector titer compared to Triton X-100. The highest costs in the viral vector production process arise from downstream processing, therefore understanding and optimizing unit operations in downstream processing is crucial to minimize product losses during the process [1]. The viral vector may denature and unfold because of shear stress and adsorption to surfaces during downstream manufacturing process [2]. Vector capsid aggregation can also significantly reduce yield. Optimization of the cell lysis process to minimize viral vector aggregation, unfolding and precipitation not only improves the viral vector titer, but it also reduces the burden on subsequent downstream unit operations to remove virus aggregates and precipitate from the final product. Since the cost of gene therapy treatment is very high, any improvement in titer will improve the cost of gene therapy production and treatment cost to the patient [3]. J.T.Baker® Cell Lysis Solution is also biodegradable according to OECD 301 guidelines for readily biodegradable material.

REFERENCES

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