# Novel Cell Lysis Solution:
# Scaling up the harvest process while preserving what matters

Despite unique manufacturing challenges, gene therapy holds great promise to prevent, treat or cure certain inherited disorders and diseases . As such, the number of therapeutics in development continues to grow; the FDA and the European Medicines Agency predict they will each approve 10 to 20 gene and cell therapies per year by 2025.[1]

Viruses such as recombinant adeno-associated virus (AAV) and lentivirus serve as the primary delivery vehicle for treatment, either by replacing or turning off defective genes, or through the introduction of new genes to treat or cure a disease.[2] In recent years, advancement in cell line development, such as suspension HEK293 or Sf9, has enabled the industry to push the AAV production scale to new heights. This upward trend has created new challenges, such as fluid handling and cell lysis at 200L or above.[3]

There are key points in the viral vector workflow that present significant hurdles:

**Upstream**

- Optimization of plasmid ratios, amount of total DNA, transfection ratio to total DNA ratio, media conditions, etc. to ensure cell transfection efficiency; thereby mitigating the high cost of the DNA and transfection reagents.
- Effective lysing of the host cell while avoiding shear stress that results in product loss.
- Scalable cell lysis and host DNA removal at the large-scale.

**Downstream**

- Product loss due to removal of empty/partially full capsids during purification and an additional chromatography step.
- AAV serotypes may require alternate purification methods.

**Fill/Finish**

- Surface adsorption (glass, stainless steel, container/closure) during concentration via tangential flow filtration (TFF), formulation and sterile filtration.

This article will not focus on all of the obstacles associated with the workflow, but on optimizing the cell lysis step using a novel detergent. A novel cell lysis solution was developed to address industry concerns over the environmental impacts of the current industry standard, as well as to improve the efficiency of viral vector release. At 100-times concentration, this ready-to-use solution requires no refrigeration and can be diluted to customer needs.

## HOW CELL LYSIS FITS IN THE PROCESS

During cell lysis, the goal is to disrupt the lipid bilayer of the cell membrane in order to release both its cellular contents along with viral particles. There are different ways to accomplish this, but typically, they consist of both physical and chemical or detergent-based methods. (Figure 1)

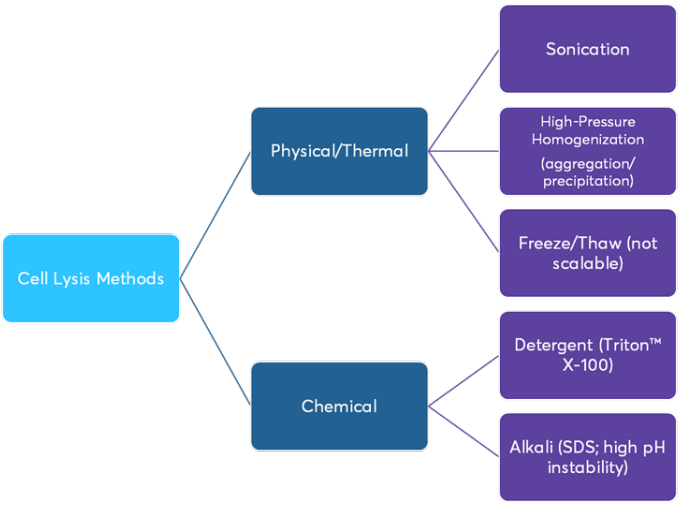


**Figure 1:** Traditional methods of cell lysis.

Each of these traditional methods has its own drawbacks. Some methods, such as freeze/thaw, are not scalable, and some present the risk of actually damaging the viral vector itself.

Detergent-based cell lysis methods work by forming micelles and solubilizing cellular membrane proteins, thus allowing release of cellular components and the viral particles of interest. Traditional detergents, however, have their own process challenges.

Consider that Triton™ X-100, long considered the industry's detergent of choice based on its superior performance, is now on the 'substance of very high concern list' by the European Chemicals Agency. In addition, Triton X-100 and detergents such as Polysorbate 20 alone do not prevent aggregation due to shear stress during processing.

Clearly, the ecotoxicity concern of Triton X-100 creates a void in the cell lysis step. The industry required a solution that would not only lyse the cells, but also protect the viral vector from damage during processing.

Switching to an alternate detergent has not been the ideal solution, as the viscous nature of commonly used detergents meansthey typically require predilution and can be process intensive to prepare, often requiring filtering and repackaging before they can be used.

## DETERGENT-BASED CELL LYSIS SOLUTION: CRITICAL PARAMETERS & STUDY RESULTS

To show proof of efficacy and compliance, we tested this novel solution and other detergents including polysorbate 20 (PS 20) and Triton X-100 at various concentrations in relation to critical parameters:

- Complete cell lysis for viral vector recovery
- Minimal impact on viral vector integrity
- Clearance during downstream processing
- Compatibility with endonuclease
- European Chemical Agency and REACH compliant

## COMPLETE CELL LYSIS FOR VIRAL VECTOR RECOVERY

A cell lysis solution should be capable of lysing healthy, untransfected cells in comparison to other commonly used detergents at typical usage concentrations, within a reasonable duration of time and across a variety of cell types and cell densities. (Figure 2).

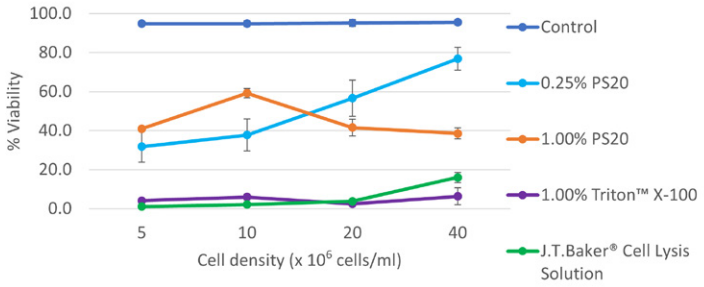


Figure 2. Viability of HEK293F cells at specified cell densities following one hour of cell lysis; as measured using a Vi-Cell XR with trypan blue exclusion staining.

As temperature and pH can influence the lysis efficiency of detergents, we tested for both variables to determine final efficacy. (Figures 3 and 4). pH is especially critical because DNA degradation with endonuclease is commonly performed at the same time as cell lysis, and the optimal pH value for endonuclease activity is 8.

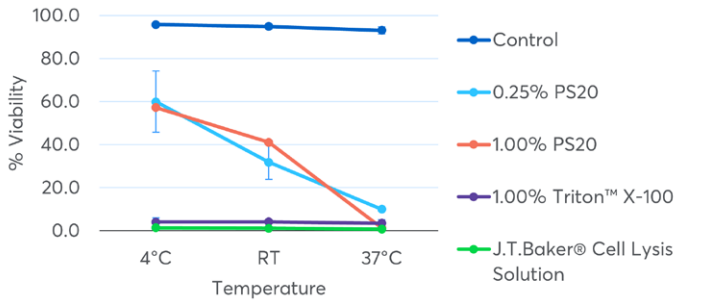


Figure 3. Viability of 5e6 HEK293F cells following one hour of cell lysis incubated at various temperatures.

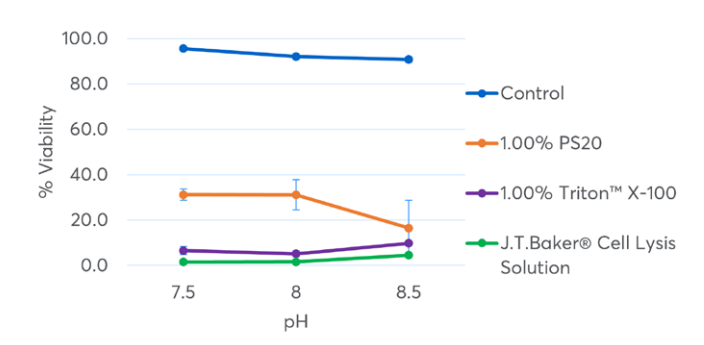


Figure 4. HEK293F cells were aliquoted, and media adjusted to the specified pH values. Lysis efficiency was calculated following one hour of detergent incubation.

This data shows that using an appropriate cell lysis solution can effectively and rapidly release the intracellular components across a range of cell densities, and at standard process temperatures and pH levels, to support flexible process development.

## MINIMAL IMPACT ON VIRAL VECTOR INTEGRITY

Once the viral particles are released from the cell during the lysis process, it is critical that the detergent lysis step does not have any effect on the integrity or yield of the released viral particles — particularly from shear stress due to agitation. This shear stress and resulting viral particle damage can lead to a decrease in downstream yield, and low yields can create a dosing problem.

If the vector concentration in a gene therapy batch is too low, developers would have to increase the dose volume to an unreasonable level. According to one study, developers must concentrate their AAV batches 100-10,000 times to reach an appropriate titer. (2)

To test capsid recovery, cell lysis was conducted at 125 RPM and 37 °C on a platform shaker, with samples taken at both two- and twenty-four-hour time points in order to harvest the cells for ELISA and ddPCR viral genome titer quantification (Figures 5 and 6).

Results showed similar titer recovery (vg/ml) post agitation between the cell lysis solution, no-detergent control, Triton X-100, and Polysorbate 20 at the tested concentrations. The data suggests J.T.Baker® Cell Lysis Solution does not cause titer loss during chemical lysis and agitation.

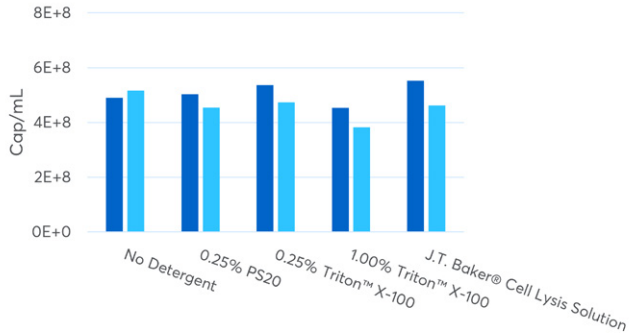


Figure 5. Viral titer measured using ELISA (Progen® AAV2 ELISA Kit) at two timepoints.

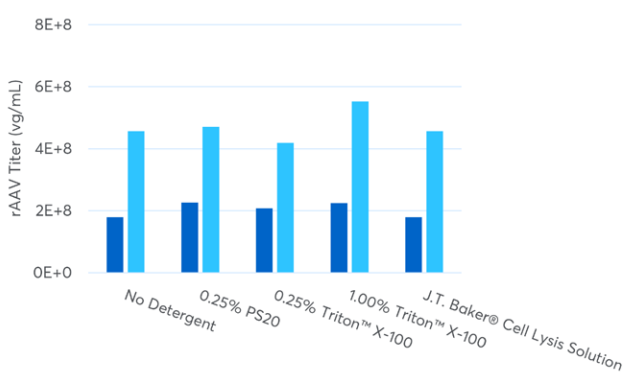


Figure 6. Viral titer measured using ddPCR at two timepoints.

## ENDONUCLEASE COMPATIBILITY

The function of endonuclease during the lysis step is critical for DNA removal of undigested plasmid DNA as well as host cell DNA released during the cell lysis step. Therefore, it is essential that the detergent of use is compatible with this enzyme.

To that end, an endonuclease activity assay was performed to determine if detergent incubation produced any negative effect on enzyme activity — both at the typical 1:100 dilution and at progressively worst-case scenarios in terms of higher detergent concentrations, such as 1:50 and 1:25 dilutions (Figure 7).

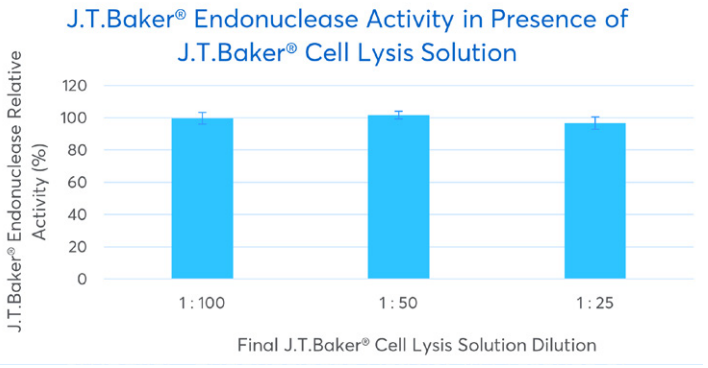


**Figure 7.** Percent endonuclease activity measured in solutions diluted as shown

Relative to no-detergent control, J.T.Baker® Endonuclease retained enzymatic activity following incubation, even at the highest concentration of the cell lysis solution tested, which enabled robust DNA clearance.

In summary, these study results demonstrate that a novel, detergent-based cell lysis solution offers comparable cell lysis performance to Triton X-100. Its consistency across a range of standard process temperatures enables flexibility and robustness during the manufacturing process, while its efficiency at standard upstream pH values enhances compatibility with endonuclease activity.

The novel cell lysis solution also provides favorable recovery of viral vectors during lysis, resulting in quality, potent vectors following downstream processing.

As a readily biodegradable and environmentally friendly formulation that clears during downstream processing, the solution provides a REACH-compliant alternative for detergent-based cell lysis.

## REFERENCES


1 Scudellari M. How gene therapy overcame high-profile failures. Science News [Internet]. 2022 Mar 22. Available from: https://www.sciencenews.org/article/gene-therapy-history-high-profile-failures.

2 White M, Alsarraj M. Quantifying AAV Viral Titer and Integrity with ddPCR. American Pharmaceutical Review [Internet]. 2021 Aug 26. Available from: https://www.americanpharmaceuticalreview.com/Featured-Articles/578724-Quantifying-AAV-Viral-Titer-and-Integrity-with-ddPCR/

3 Stamatis C, Chatel A, Farid S. Can novel bioreactors improve the cost of goods of viral vectors? Cell & Gene Ther Ins. 2023; 9(5), 687–704.


## ABOUT THE AUTHOR:


**Beth Kroeger-Fahnestock**
**Director, New Product Introduction (BioPharma), Avantor, Inc.**


Beth Kroeger-Fahnestock is a Director, New Product Introduction, in Avantor's Biopharma Production division. She currently manages new product launches to support Avantor's mission in bringing lifesaving medicines to market. She has extensive industry experience in Manufacturing, Validation, Technical Transfer, R&D, Compliance, and Quality from her various positions she has held. Her areas of expertise include large-scale Bioprocess systems, downstream purification operations, and Process and Cleaning Validation along with cleanroom environmental control, speaking frequently on these topics for educational and professional organizations. She served on the ISPE task force responsible for writing the ISPE Guidance: Cleaning Validation Lifecycle – Applications, Methods, and Controls Good Practice Guide, published in 2020 and was an Adjunct Lecturer, Temple University, School of Pharmacy, RA/QA Graduate Program for several years. She earned a B.S. in Biochemistry from the University of Missouri, St. Louis.