Novel Cell Lysis Solution: Scaling up viral vector harvest with ease and regulatory compliance

INTRODUCTION

The promise of gene therapy is driving an unprecedented surge in clinical trials, with over 600 in progress, accounting for approximately 32% of the Cell & Gene Therapy (CGT) clinical trial portfolio.[1] Many of these gene therapies use viral vectors as delivery vehicles to replace or knock out target genes or introduce new genes to treat a disease. Viruses like adeno-associated virus (AAV) and lentivirus (LV) are examples of these vectors and are widely used as drug products or in the manufacturing process in CGT modalities.

Due to strong demand and recent advancements into non-rare diseases in clinical trials, many manufacturers are looking to scale up their production for AAV and LV.[2] Supported by optimized cell lines, such as suspension HEK293 or Sf9, an emerging trend is to scale the bioreactor volume, in some cases above thousands of liters.[3] However, this new trend also comes with new challenges, such as fluid handling and cell lysis at 200 L or above.[4]

CELL LYSIS FOR LARGE-SCALE BIOPRODUCTION

There are many complex steps in AAV/LV manufacturing processes, especially during transfection and purification. When the production scale increases, the operations and logistics of cell lysis and harvest also become significantly more challenging. In the bioproduction environment, chemical cell lysis with detergents has been favored due to its scalability and lower cost. Triton™ X-100, long considered the industry's detergent of choice, is now on the 'substance of very high concern list' by the European Chemicals Agency (ECHA). The restrictions placed on this detergent created a void in the cell lysis step. As a result, the industry required a replacement that also meets new manufacturing needs. Considering the essential requirements for cell lysis and the harvest step, a novel cell lysis solution was developed that is effective, scalable, easy to use and regulatory compliant. This article will discuss the ideal cell lysis reagent in a bioproduction environment (Figure 1) and how J.T.Baker® Cell Lysis Solution (CLS) compares.

ATTRIBUTES OF AN IDEAL CELL LYSIS REAGENT

Ease of Use

+ Ease of fluid-handling
+ Ease of operation and logistics
+ Compatibility with endonuclease for DNA digestion

Regulatory Compliance

+ European Chemicals Agency and REACH compliant

Performance

+ Lysis efficiency and viral vector recovery
+ Minimal impact on viral vector integrity
+ Clearance during downstream processing

FIGURE 1. Attributes of an ideal cell lysis reagent for bioproduction applications are ease of use, regulatory compliance and performance. Product features and data in each category are discussed in detail in this article.

NOVEL DETERGENT-BASED CELL LYSIS SOLUTION: EASE OF USE

To illustrate ease of use, the viscosity of this novel solution was compared with the stock solution of generic detergents like Triton X-100. Stored at room temperature, Triton X-100 typically has a viscosity of around 240 centipoise (cps), whereas CLS has a viscosity of around 8 cps. This low viscosity makes transferring and measuring volume a much easier task in the production environment.

At 100X concentration, the required volume for CLS is only one one-hundredth of the cell culture volume. Compared to generic detergents, which are typically prepared at 10X concentration, CLS presents fewer fluid handling challenges and saves significant bioreactor headspace in large-scale viral vector productions. Its ready-to-use format reduces preparation time and minimizes the potential for inconsistencies, further streamlining the harvest step.

Compatibility with endonuclease for DNA digestion, a common process step after cell lysis, was evaluated as a final proof for ease of use. 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 and further processing steps to maintain purity and quality.

To test if endonuclease can be used without a buffer exchange or similar process step, 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 2). Relative to the 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.

NOVEL DETERGENT-BASED CELL LYSIS SOLUTION: REGULATORY COMPLIANCE

Prompted by environmental and health concerns, the ECHA has established restrictions on chemicals (known as REACH), which lists Triton X-100 as a substance of very high concern (SVHC). This restriction is important to biopharma firms globally due to harmonization of procedures, redundant manufacturing for business continuity planning and import considerations. It's also important globally since it raises a concern about future regulatory restrictions in other countries. As a result, biologics manufacturers are now moving away from Triton X-100 to comply with EA regulations or to future-proof their manufacturing process. Unlike industry-standard products that often contain solvents with phosphates and endocrine-disrupting detergents, J.T.Baker Cell Lysis Solution is free of

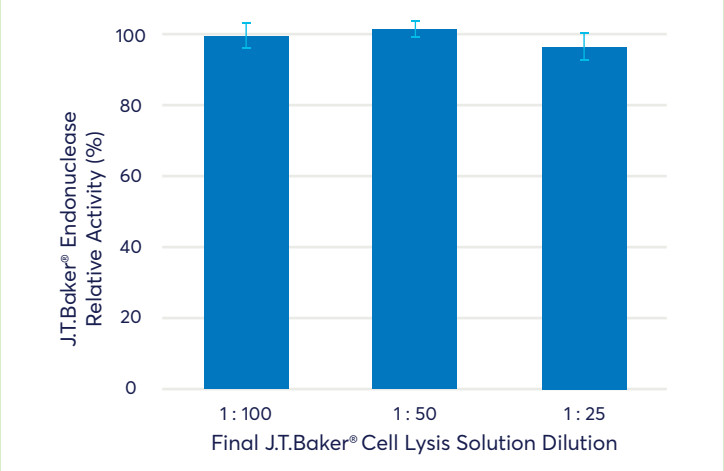

FIGURE 2. Data shows that in the presence of J.T.Baker Cell Lysis Solution at 1:25, 1:50 and 1:100 dilution factors, J.T.Baker Endonuclease retains above 95% enzymatic activity and showed DNA clearance in internal testing.

alkylphenols and has low aquatic toxicity, making it safer for both users and the environment. The waste product is environmentally friendly, ensuring minimal impact on ecosystems. Because of these attributes, this product is a regulatory-compliant replacement for Triton X-100 for cell lysis applications.

NOVEL DETERGENT-BASED CELL LYSIS SOLUTION: PERFORMANCE

To evaluate performance, this novel solution and other detergents, including polysorbate 20 (PS 20) and Triton X-100, were tested at various process conditions in relation to critical parameters:

- Lysis efficiency and viral vector recovery
- Minimal impact on viral vector integrity
- Clearance during downstream processing

Lysis efficiency and 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, with fast reaction time and across a variety of cell types and cell densities (Figures 3a and 3b). As temperature and pH can influence the lysis efficiency of detergents, we tested for both variables to determine final efficacy (Figures 3c and 3d). 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. 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.

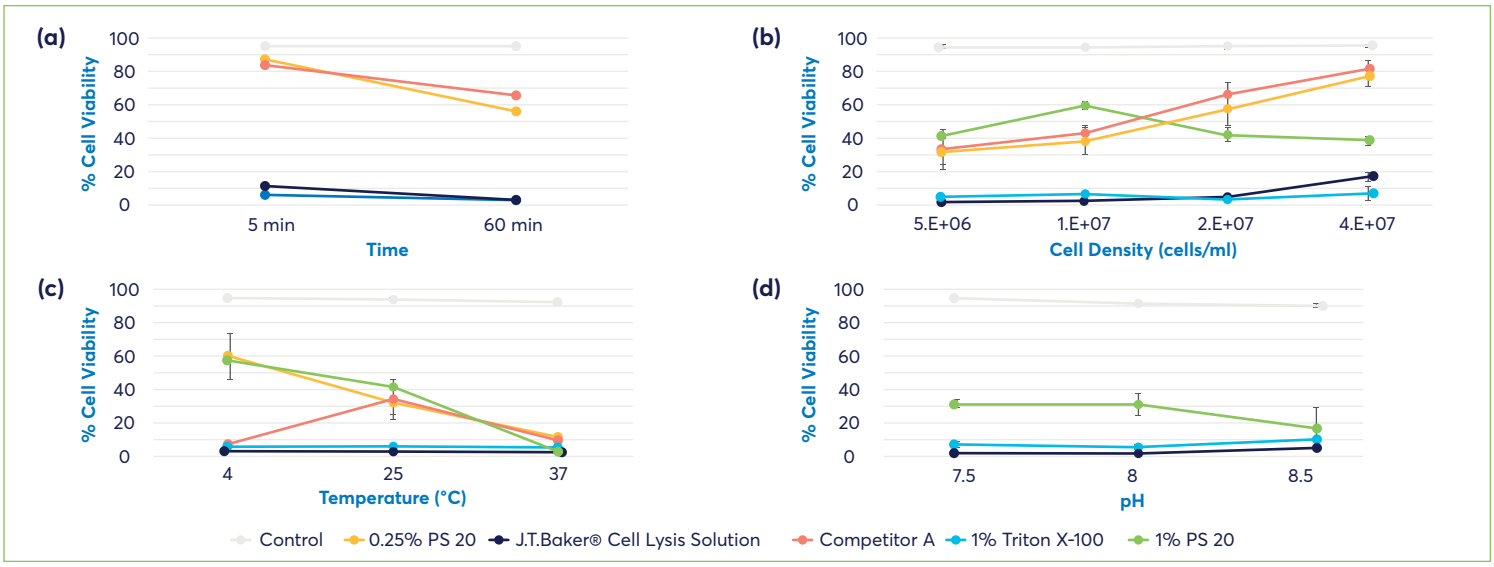

FIGURE 3. Performance data shows J.T.Baker Cell Lysis Solution is comparable or superior to market options across several benchmark dimensions: (a) viability of HEK293 cells at 20E6/mL following cell lysis for 5 versus 60 minutes (b) viability of HEK293F cells at specified cell densities following one hour of cell lysis (c) viability of 5e6 HEK293F cells following one hour of cell lysis incubated at various temperatures (d) HEK293F cells were aliquoted, and media adjusted to the specified pH values. Lysis efficiency was calculated following one hour of detergent incubation. All cell viability data was measured using a Vi-CELL XR with trypan blue exclusion staining.

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.[5]

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 4a and 4b). Results showed similar titer recovery (cap/ml and 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 that CLS does not cause titer loss during chemical lysis and agitation in a typical process or overnight incubation.

Clearance during downstream processing

A key consideration in detergent-mediated cell lysis is ensuring that the subsequent process steps can remove the detergent from the product. This removal is crucial for maintaining sample integrity, ensuring the smooth progression of experiments and adhering to regulatory standards. Typically, residual detergent is removed through various purification techniques, such as

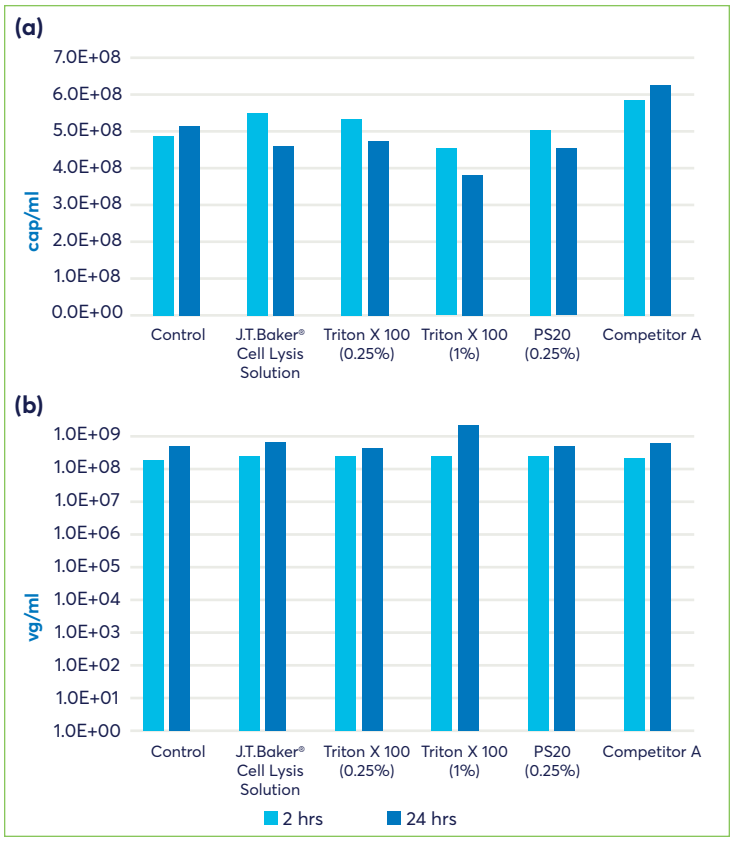

FIGURE 4. Benchmark data on viral particle recovery after 2 and 24-hour incubation, suggesting J.T.Baker Cell Lysis Solution behaves similarly to other market options. (a) Viral titer measured using ELISA (Progen® AAV2 ELISA Kit) at two time points. (b) Viral titer measured using ddPCR at two time points.

chromatography, filtration or diafiltration. Anion exchange (AEX) chromatography is often used as a polishing step in AAV processes to remove residual impurities. Therefore, a comprehensive study has been conducted to demonstrate CLS clearance capability of AEX resin.

To examine the detergent clearance capability, a high-performance liquid chromatography (HPLC)-based analytical method was devised to detect active detergents present in samples treated with CLS. The evaluation involved spiking a 1% CLS solution into the load sample, which was then processed using AEX chromatography on a J.T.Baker® BAKERBOND® PolyQUAT™ column (SN# 7602). The run parameters are summarized below (Table 1). Detergent content in all samples was analyzed using an HPLC detection method.

TABLE 1. Protein A chromatography process parameters

Process parameters	Description
Resin	J.T.Baker BAKERBOND PolyQUAT
Column volume (CV)	1 ml (3.3 cm B.H. x 0.6 cm I.D.)
Residence time	3 min (flow rate: 0.33 ml/min)
Equilibration	25 mM Tris, pH 7.2, 10 CV
Load	1% Cell Lysis Solution in 49.5 ml Milli-Q®2 water
Wash 1	Water, Type 1, 18.2 MΩ cm at 25 °C, Milli-Q
Wash 2	25 mM Tris, pH 7.2 (Buffer A)
Elution	25 mM Tris, 1M NaCl pH 7.2 (Buffer B) Linear gradient 0 - 100% Buffer B
Strip	25 mM Tris, 1M NaCl, pH 7.2
CIP	0.5 N NaOH

The analytical data indicated excellent detergent clearance capability of AEX chromatography, as the detergent amount in protein A eluate was below the limit of detection (<0.01%) by the HPLC method. There was no visible detergent peak in the analytical HPLC chromatogram (Table 2).

TABLE 2. Detergent clearance by J.T.Baker BAKERBOND PolyQUAT column chromatography

Sample	Total concentration
Load	1% J.T.Baker Cell Lysis Solution
Elution	<0.01% J.T.Baker Cell Lysis Solution

CONCLUSION

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

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