Technical note

J.T.Baker® Cell Lysis Solution Cell Lysis Efficiency

Cells have a lipid bilayer membrane that protects and regulates permeation into the cell. Cell lysis methods are commonly used in downstream processing to isolate or release intracellular materials such as DNA, RNA, proteins and viral particles. In the case of viral vector production for cell and gene therapy, certain adeno associated viral (AAV) serotypes are not easily released from the cell, and the cells must be ruptured to release the viral vector particles for further purification. Chemicals such as Triton™ X-100, sodium deoxycholate, and sodium dodecyl sulfate (SDS) have been used as primary detergents for cell lysis [1]. This method of cell disruption is less harsh on the protein or viral vector particles being extracted than physical methods, such as freeze thaw or homogenization. Detergents work by forming micelles that solubilize proteins in the lipid bilayer of the cell membrane, rupturing the cell to release non-enveloped viral particles. Triton X-100 has been the preferred non-ionic detergent for viral vector purification due to its superior performance [1]. However, research has shown that Triton X-100 causes acute oral toxicity, eye damage, skin irritation, and chronic aquatic toxicity [2]. As a result, the detergent has been designated a "substance of very high concern" by the European Chemicals Agency's Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulations, in December 2012 [3]. The detergent has been banned for use in the pharmaceutical industry in Europe since January 2021 and other regions of the globe may follow [4]. In these studies, the effectiveness of J.T.Baker® Cell Lysis Solution to lyse cells was compared to Triton X-100 for two cell lines, HEK293F and SF9, as well as to other non-ionic detergents for HEK293F.

MATERIALS AND METHODS

In order to evaluate the effectiveness of the J.T.Baker® Cell Lysis Solution, cell lysis experiments were performed. In the first study, lysis of two cell lines was compared at different concentrations of detergent. Expi293F cells (Gibco, A14527) were grown in Expi293 expression medium (Gibco, A14351-01) in a vented non-baffled shake flask at 37°C, 125 rpm, 6% CO2, and >60 relative humidity. SF9 cells (Gibco, 11496015) were grown in SF-900 II SFM (Invitrogen/Gibco, 10902) media in a non-vented non-baffled shake flask at 27°C and 125 rpm. The target cell count of >5x106 viable cells/ml was achieved in 4-5 days for both cell types. 2 ml of cells in media were added to each of 10 x 15 ml tubes under a sterile biosafety cabinet. Each tube was spiked with 0% (control, no detergent), 0.05%, 0.10%, 0.25%, and 0.5% final detergent concentrations (w/v%) of either Triton X-100 or J.T.Baker® Cell Lysis Solution using concentrated stocks. Cell counts and percent cell viability were assessed after 1 hour of incubation at ambient temperature using the Vi-Cell XR cell viability analyzer (Beckman Coulter). The Vi-Cell XR Cell Viability Analyzer uses a trypan dye exclusion method to measure cell viability and cell concentration. Upon automated mixing of trypan blue and the cells at 1:1, live cells (no dye uptake) versus dead cells are counted in the flow cell, via automated imaging software. In the second study, cell lysis of HEK293F cells with J.T.Baker® Cell Lysis solution at the recommended 1:100 dilution was compared to three other non-ionic detergents at 0.1% final detergent concentration using the same experimental protocol.

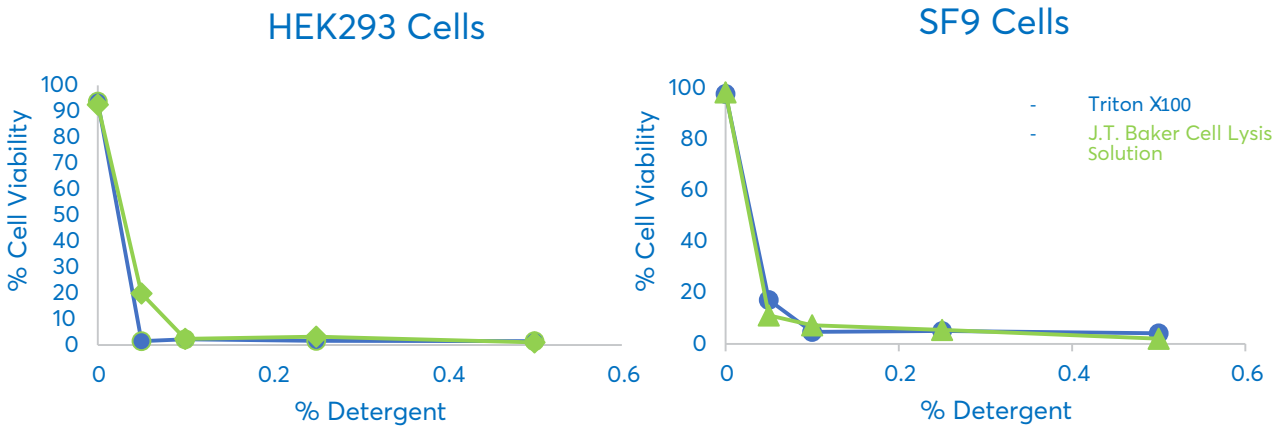

Figure 1: Lysis of HEK293 and SF9 cells using Triton-X100 and Cell lysis solution after 1 hr incubation

J.T.BAKER® CELL LYSIS SOLUTION EFFICIENCY

Efficiency of lysis of Expi293F and SF9 cells by J.T.Baker® Cell Lysis Solution and Triton X-100 was determined at various concentrations of detergent as described. Cell viability prior to lysis was measured for both cell types. Cell viability as a function of lysis solution concentration was determined following 1 hour of incubation using the ViCell cell viability analyzer. Results of cell viability are shown in Figure 1. The percentage of cells lysed for both Expi293F and SF-9 cells was comparable for J.T.Baker® Cell Lysis Solution and Triton X-100. Complete lysis was achieved above 0.1% (v/v) final detergent cell lysis concentration, which is the typical detergent concentration used for cell lysis [4-8].

Cell lysis of HEK293F cells with J.T.Baker® Cell Lysis Solution at the recommended 1:100 dilution compared to commonly used non-ionic detergents at 0.1% final detergent concentration is summarized in Figure 2. The data shows that lysis of HEK293F cells by J.T.Baker® Cell Lysis Solution is comparable to Triton X-100 and superior to other commonly used non-ionic detergents at 0.1% and 0.5%. For Competitor B, complete cell lysis was not achieved at the concentrations tested, whereas Triton X-100 and J.T.Baker® Cell Lysis Solution showed a marked loss in cell viability at both concentrations.

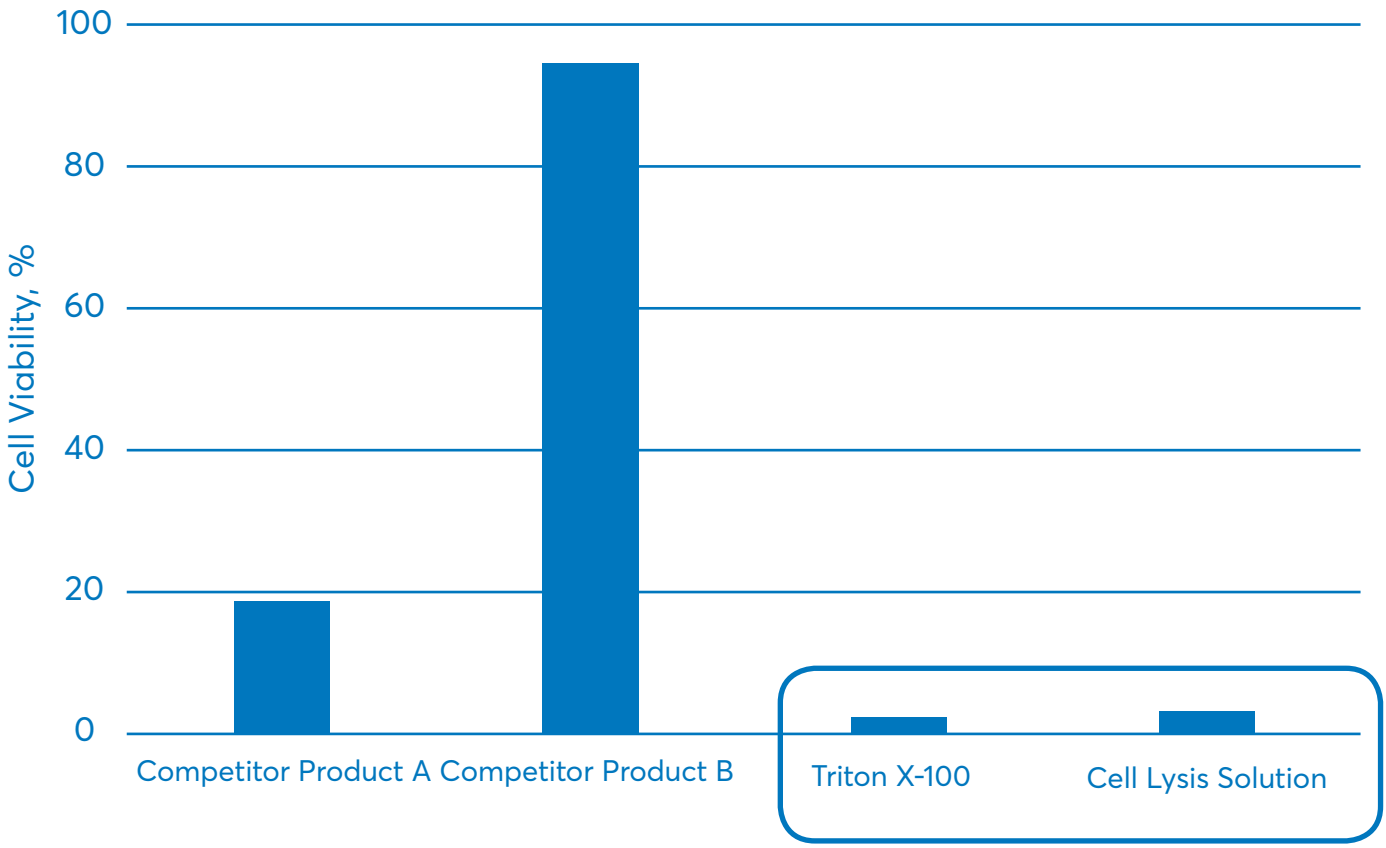

Figure 2: Viability of HEK293F cells following cell lysis using various detergents. Lysing agent mixed in cell media for 1 hour before cell extraction.

CONCLUSIONS

There are several detergents which are commonly used to lyse cells. This is an important initial step in the isolation of proteins, DNA and viral vector particles from cells. These studies showed that J.T.Baker® Cell Lysis Solution displays comparable lysis efficiency compared to other non-ionic detergents such as Triton X-100, and can be used for several cell lines commonly used for protein and AAV vector production. J.T.Baker® Cell Lysis Solution is an alternative to Triton X-100 that is ready to use and biodegradable according to OECD 301 guidelines for readily biodegradable materials.

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