J.T.Baker® Cell Lysis Solution Detergent Clearance using Anion-exchange Chromatography

OBJECTIVE

J.T.Baker® Cell Lysis Solution Biotech Reagent is a cGMP-compliant, ready-to-use product featuring a proprietary blend of biodegradable[1] nonionic surfactants. It is specifically designed to extract viral particles, including Adeno-associated Virus (AAV) particles, from HEK293 and SF9 cells while preserving viral capsid integrity during the lysis process. This innovative formulation is not listed on the REACH SVHC (Substances of Very High Concern) list and is free of alkylphenol ethoxylates. It is available in various sizes to support processes from development to large-scale commercial batches. Additionally, this solution does not interfere with endonuclease activity, allowing for effective cell lysis and simultaneous DNA/RNA degradation in a single step.

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 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 J.T.Baker® Cell Lysis Solution clearance capability of AEX resin

METHOD

Examining the detergent clearance capability requires a reliable analytical tool to track residual detergent in materials post-purification. Consequently, a High-Performance Liquid Chromatography (HPLC)-based analytical method was devised to detect active detergents present in samples treated with J.T. Baker® Cell Lysis Solution (CLS). J.T.Baker® Cell Lysis Solution detection method, Analytical Method 200-300-1001, is accessible under an active Confidentiality/Non-disclosure Agreement.

The evaluation involved spiking a 1% CLS solution into the load sample, which was then processed using anion-exchange (AEX) chromatography on a J.T.Baker® BAKERBOND® PolyQUAT column (SN# 7602). The run parameters are summarized in 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 (flowrate: 0.33 mL/min)
Equilibration	25mM 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	25mM Tris, pH 7.2 (Buffer A)
Elution	25mM Tris, 1M NaCl pH 7.2 (Buffer B)

	Linear gradient 0- 100% Buffer B
Strip	25mM Tris, 1M NaCl, pH 7.2
CIP	0.5N NaOH

RESULTS AND DISCUSSION

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 analytical HPLC chromatogram. Table 2 represents the detergent clearance data by J.T.Baker® BAKERBOND® PolyQUAT column.

Table 2: Detergent clearance by J.T.Baker® BAKERBOND® PolyQUAT column chromatography

Sample	Total Concentration
Load	**1% CLS**
Elution	**<0.01% CLS**

CONCLUSION

In biologics manufacturing, a critical consideration when choosing a detergent for cell lysis is ensuring that subsequent process steps can efficiently remove the detergent from the intermediates. The study data clearly demonstrate that the detergent is reduced to levels below 0.01% using AEX chromatography. Based on the study data and the physicochemical properties of the detergent, it can be concluded that this detergent can be readily eliminated through ion-exchange chromatography.

1. ISO 7827:2010, OECD Test Guideline 301F (28d): >60% (10-day window met)
2. Milli-Q® is a registered trademark of MERCK KGAA