J.T.Baker® Cell Lysis Solution Detergent Clearance using Affinity Chromatography

OBJECTIVE

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

One of the critical aspects of using detergent-mediated cell lysis is that the subsequent process step is able to remove the detergent from product. Removal of detergent is pivotal for maintaining sample integrity, ensuring smooth progression of experiments, and adhering to regulatory standards. Typically, residual detergent removal is accomplished through diverse purification techniques like chromatography, filtration, or diafiltration, Therefore, a comprehensive study has been performed to demonstrate the detergent clearance capability of an AAV affinity chromatographic resin.

METHOD

To investigate the detergent clearance capability, a suitable analytical tool is essential for tracking residual detergent in materials after they undergo a subsequent purification step. Therefore, a High-Performance Liquid Chromatography (HPLC)-based analytical method was developed to detect the active detergents of J.T. Baker® Cell Lysis Solution (CLS) in sample. J.T.Baker® Cell Lysis Solution detection method, Analytical Method 200-300-1001, is available under an active Confidentiality/Non-disclosure Agreement.

The evaluation was executed by spiking 1% CLS in load sample and processed over a Cytiva Capto™[2] AVB column (PN# 17372211). The run parameters are summarized in **Table 1.** Detergent content in all the samples were analyzed by HPLC detection method.

Table 1: Protein A chromatography process parameters

Process parameters	Description
Resin	Cytiva Capto™ AVB
Column volume (CV)	1 mL (2.5 cm B.H. x 0.7 cm I.D.)
Residence time	2 min (flowrate: 0.5 mL/min)
Equilibration	20mM Tris, 0.5M NaCl pH 7.5, 10 CV
Load	1% CLS in 20mM Tris, 0.5M NaCl, pH 7.5

Wash 1	20mM Tris, 0.5M NaCl pH 7.5, 10 CV
Elution	100mM Sodium Acetate, 0.5M NaCl, pH 3.0
Strip	100mM Phosphate, 0.5M NaCl, pH 1.7

RESULTS AND DISCUSSION

The analytical data indicated excellent detergent clearance capability of protein A 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 Cytiva Capto™ AVB column.

Table 2: Detergent clearance by Cytiva Capto™ AVB column chromatography

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

CONCLUSION

A crucial factor in choosing a suitable detergent for cell lysis in biologics manufacturing is ensuring that subsequent process steps can effectively remove the detergent from the intermediates. The study data clearly show that the detergent is removed to a level of less than 0.01% through AAV affinity chromatography. Based on the detergent's physicochemical properties, it can be inferred that this detergent can be easily removed by any affinity, ion-exchange or other chromatography processes using a bind-and-elute mode of operation.

1. ISO 7827:2010, OECD Test Guideline 301F (28d): >60% (10-day window met)
2. Capto is a trademark of Cytiva. Cytiva and the Cytiva Drop Logo are the sole and exclusive property of Life Sciences IP Holdings Corporation or an affiliate thereof. All other trademarks are the property of their respective owners.