

DAISEP MabXpure™, a single-use multimodal (SEC-AEX) flowthrough (FT) technology application offering a high performance solution for the depletion and reduction of Host Cell Proteins (HCPs) and DNA following clarification/harvest, during purification, polishing and sample preparation of MABs and large molecules.



INTRODUCTION

Today, biopharmaceutical manufacturing has shown considerable improvements in upstream processing efficiencies, enabling high process titers in excess of 10 g/L associated with high cell densities ($>60 \times 10^6$ cells/mL). This increase in productivity also creates high levels of process-related impurities like higher HCP levels ($>800,000$ ng/mL), host cell DNA (>1000 ng/mL) content, HMW, fragments and/or lipids. The huge increase in bioburden and impurity levels shifts the production bottleneck to purification (clarification, chromatography and filtration).

In the current scenario, primary separation techniques like centrifugation, depth filtration combined with secondary purification operations are reaching their limits. Conventional bioseparation technologies are unable to provide an efficient, consistent and sustainable purification platform in terms of impurity reduction and/or bioburden depletion.

Recently, there has been considerable focus on the development of new single-use flowthrough technologies since they can provide a unique flexibility of being easy to

implement with excellent process performance and achieving high process recoveries. DAISEP MabXpure is a pre-filled disposable device which is developed based on the aforementioned performance criteria to ensure significant bioburden depletion using a combination of anion exchange (AEX) and size exclusion (SEC) mechanisms.

DAISEP MabXpure is designed to trap various impurities primarily targeting bioburden in the clarified cell harvest and bulk drug substance process intermediates. These impurities and bioburden are considerably reduced or eliminated whilst being retained by the resin and the mAbs are recovered in the flowthrough (Figure 1). The resin is packed in an integrated format which has been designed to be a robust scalable plug and play device (Figure 2).

DAISEP MabXpure's high retention capacity for impurities and bioburden allows for high depletion rates of HCP and DNA $>75\%$ from the process feed/bulk drug substance intermediates. MabXpure has a high selectivity for co-eluted HCP traces, and simultaneously ensures high recovery rates of the target molecules and mAbs ($>95\%$), delivering a much higher quality drug substance or intermediate product in the manufacturing process conventional and current purification technologies.

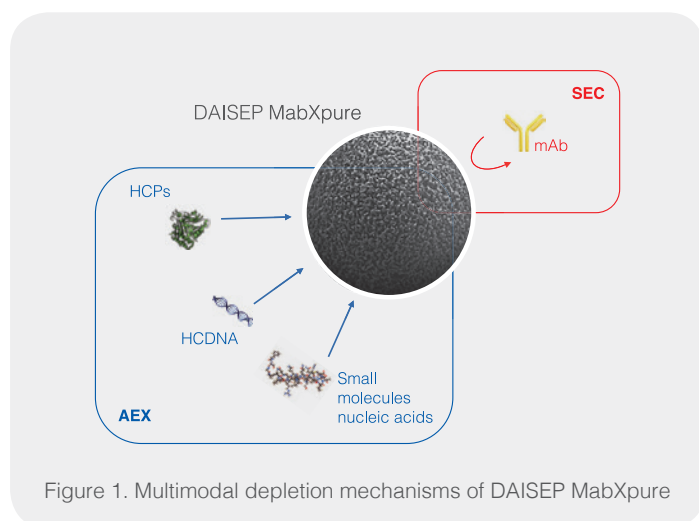


Figure 1. Multimodal depletion mechanisms of DAISEP MabXpure



Figure 2. MabXpure cartridge

MATERIALS AND METHODS

LOAD MATERIAL

CHO-K1 clarified feed,
mAb 2g/L

BUFFER

Tris HCl 50 mM pH 7.4

DEVICE

MabXpure prepacked

Depletion testing in the dynamic mode is performed using the CHO-K1 process feed flow injection through MabXpure cartridge. Collection of 5 column fractions (CVs) is performed with different residence times. Following this flowthrough process, quantification of HCP is performed using the CHO-

specific ELISA kits from Cygnus and mAb titer is measured by HPLC-SEC. Additional pooled fraction analysis was performed to compare the bioburden depletion with a 1:20 mix ratio (resin to product volumetric ratio) under static conditions.

RESULTS

In conventional AEX flowthrough applications, i.e., chromatography membranes and other AEX resins, the presence of divalent phosphate ions in the feed limits the ion exchange mechanism, i.e., the adsorption capacity of the technology for acidic HCP and impurities. However, there is a displacement effect observed after 20CVs where 75% HCPs are removed in the flowthrough and retained in the eluted fraction. Those performances of DAISEP MabXpure are consistent with the depletion levels observed under static conditions (refer to Application Note #1).

In dynamic mode, the affinity for low molecular weight and negatively charged species increases at shorter residence times where smaller impurities are forced to diffuse and penetrate into the porous structure of MabXpure and are trapped even more effectively in comparison to static process conditions. MabXpure is ideally designed for significant depletion of HCP traces from the process feed during the polishing stage. However, with DAISEP MabXpure, up to 75% of HCPs are removed under flowthrough conditions (Figure 3, pool fraction – 20 CVs) and recovery yield is >95% (Figure 4).

HCP Depletion Performances vs residence time

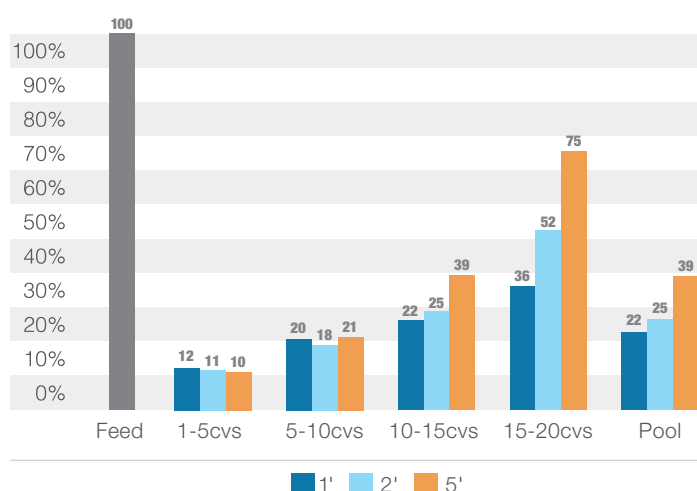


Figure 3. Residual HCP with 3 different residence times with DAISEP MabXpure.

mAb Recovery Performances vs residence time

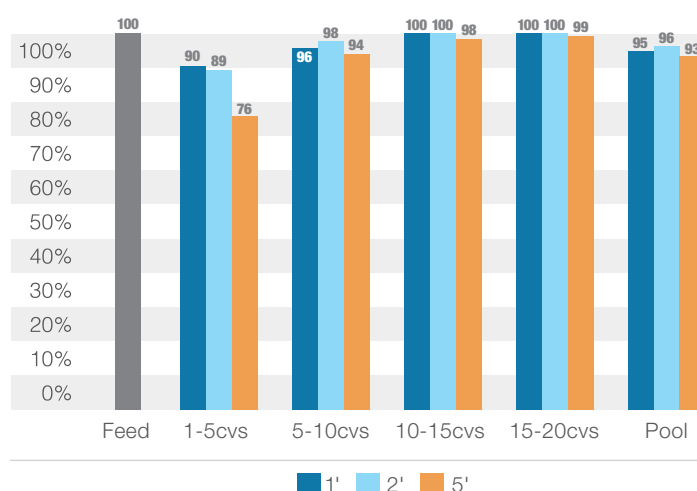


Figure 4. mAb recovery for 3 residence times with DAISEP MabXpure.

DISCUSSION

In its pre-filled resin filter cartridge (RFC) design, the DAISEP MabXpure fits very easily within a platform purification process where flowthrough conditions need to be streamlined in the process following the clarification/harvest stage. Protecting the platform whilst supplementing, complimenting or replacing the existing chromatographic steps becomes easier like MabXpure which is less intrusive to the product and the process eliminating the need for a bind and elute process step operation.

DAISEP MabXpure can be easily applied to polish the process feed without negatively impacting the process performance in the presence of phosphate ions, and it does so ideally with a short contact time. Its high throughput capacity is adequate for sample preparation applications where a fast, simple and robust solution is desired for process developmental work or to find optimal conditions for in-process implementation of MabXpure.

CONCLUSION

DAISEP MabXpure has a high potential of depleting Host Cell Proteins and DNA with extremely high mAb recovery rates. Depending on the process conditions and residence times, the capacity of DAISEP MabXpure can eliminate up to 75% of HCPs with mAb recoveries >95%.

The simple design of DAISEP MabXpure (single-use pre-filled cartridge) makes it an ideal flowthrough device to protect the downstream platform as well as to polish the feed before and after various chromatographic and TFF stages in the purification process.

MabXpure can supplement, complement or replace the pre-existing DSP unit operations

DAISEP MabXpure is easily scaleable and can be applied at lab through to process GMP scale.

DAISEP MABXPURE RANGE

MABXPURE BULK

MabXpure bulk 50 mL	DMXBK0050
MabXpure bulk 500 mL	DMXBK0500
MabXpure bulk 1000 mL	DMXBK1000
MabXpure bulk 10L	DMXBK10

MABXPURE FT

MabXpure FT 1 mL (5 units/pack)	DMXFT0001
MabXpure FT 5 mL (5 units/pack)	DMXFT0005
MabXpure FT 50 mL (1 unit)	DMXFT0050
MabXpure FT 500 mL (1 unit)	DMXFT0500
MabXpure FT 1.5 L (1 unit)	DMXFT1500

MABXPURE KIT

MabXpure Kit	DMXKT0001
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DAISEP SPIN XS & XL

DAISEP Spin XS (40 units/pack)	DSPXS0040
DAISEP Spin XL (8 units/pack)	DSPXL0008



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