

DAISEP MABXPURE: INNOVATIVE STRATEGIES FOR ENHANCING HCP CLEARANCE IN CLARIFICATION AND DOWNSTREAM PROCESSING OF MABS

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INTRODUCTION

In recent years, biopharmaceutical manufacturing has shown significant improvements in antibody production, with ever-increasing titers generally associated with higher cell densities. These increases in productivity induces higher levels of process-related impurities like host cell proteins (HCP), DNA, HMW fragments or lipids and consequently shifts the production bottleneck to the downstream steps (clarification, chromatography, and filtration). In such context, centrifugation and filtration techniques have reached their limit and will not be able to provide enough protection to the downstream purification platform.

TECHNOLOGY

DOWNSTREAM

DAISEP MabXpure is used, in bulk form, as a filter

MabXpure depletes HCP and DNA, under static

conditions, by mixing the mAb-containing feed

Filtering-off the resin is required to recover a

aid for clarification/harvest.

depleted clarified feed.

DAISEP MabXpure[™], an innovative flow-through single-use technology (SUT) combines multimodal depletion mechanisms (Size Exclusion and Anion Exchange) to improve bioburden depletion performances. MabXpure can be used as a single device technology or within a platform frame. The technology can be used as a filter aid, by mixing the resin with very low ratio feed/media, or as a polishing cartridge, when prepacked, without compromising the antibody yield (>90%). As a flow-through technology, MabXpure removes up to 2 LRV for DNA and 1 LRV for HCP under static or dynamic conditions.

The flexibility of MabXpure is unrivaled. As a filter aid, it allows the combination of centrifuge and depth filtration via alluvial filtration, as a prepacked cartridge, it is used as a Protein A guard column or as ion exchange DSP step combination. MabXpure improves overall bioburden depletion and the co-eluted impurities profile before formulation.

UPSTREAM HARVEST ADF/CF CENTRIFUGE 2 **BIOREACTOR** DÄÏCEL YOUR SAMPLE PURIFIED PROTEIN

PROCESS SCHEME OF MABXPURE IMPLEMENTATION

FILL & FINISH

4 TFF CEX AEX PΑ the formulation.

Objectives: · Reinforce clarification/harvest with dedicated chromatographic techniques. • Prevent the improvements in USP to challenge the performances of the purification platform. Target the capture of co-eluted HCPs all along the process that jeopardize the stability of

SANOFI CASE STUDY



• DAISEP MabXpure FT is a pre-filled filter cartridge used for impurity capture and polishing purposes. MabXpure FT depletes HCP and DNA under dynamic conditions from a mAb-containing feed. Traces of co-eluted HCPs and remaining DNA can be removed easily under flow-through conditions.

MABXPURE AS FILTER AID (STATIC PERFORMANCE) Depletion Performances vs contact times Depletion Performances vs mix ratio (resin:sample) 80% 70% 60% 60%

50% 50% 40% 40% 30% 20% ■HCPs ■DNA ■IgG ■HCPs ■DNA ■IgG CHO-K1, DNA 1.000 ng/mL, HCP 180 µg/mL, IgG 2 mg/mL

 With classic filter aids, there is a trade-off between bioburden depletion (HCPs and DNA) and mAb recovery. MabXpure guarantees combined performance for

 Increasing contact time improves bioburden depletion. MabXpure adapts to your needs with contact time and mix ratio.

sample purification or process solutions. • >1 LRV for HCPs and 2 LRV for DNA can be Flexibility of MabXpure resides in the mix ratio to be used and the time of contact.

Depletion Performances vs IgG subclass Depletion Performances vs IgG ■HCP ■IgG ■HCP ■IgG ■DNA

■HCPs ■lgG Depletion Performances vs mAb titer (g/L) 80% -■HCPs ■IgG

Depletion Performances vs Feed pH

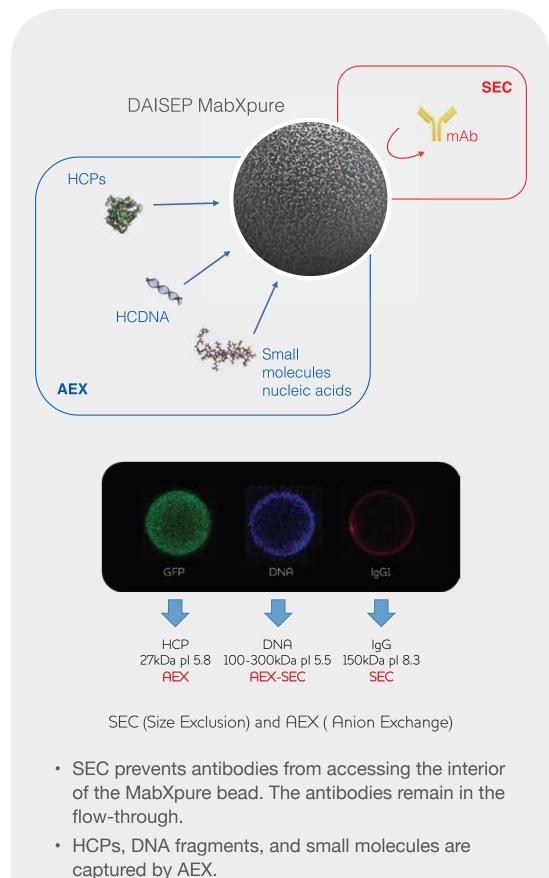
Depletion Performances vs IgG origin pH5 pH6.7 ■HCP ■IgG

 Various process conditions (e.g., titer, pH) can be used without impacting bioburden depletion and mAb recovery.

MabXpure insures a robust HCPs/DNA depletion platform. All antibodies subclasses, types and origins can be used with MabXpure. Adaptation of pH is required for low IgG's isoelectric point and balance

between HCP depletion and IgG recovery must be assessed.

MECHANISM OF INTERACTION & DEPLETION



90% 80% 70% 60% 50% 40% 30% 20% Acetate NH4OAc HCl pH 7.5 pH 5.6 pH 6.5 Virus Clearance with MabXpure FT

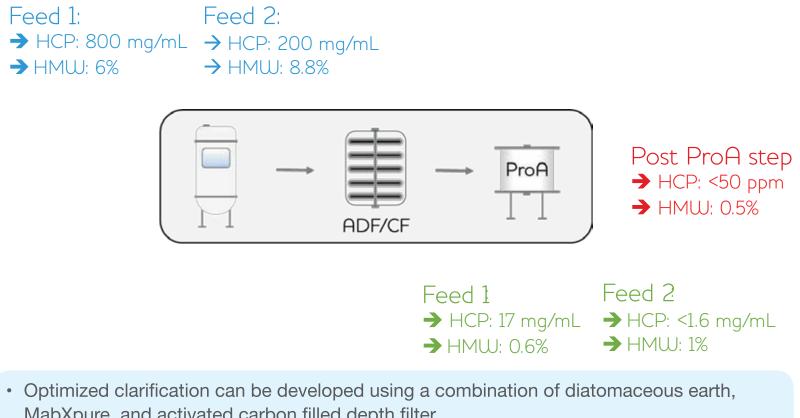
Phosphate ions do not impact the depletion. Thanks to the AEX mechanism, virus clearance can be claimed and up to 5 LRV for MVM and MuLV can be reached.

\$1,000,000

MABXPURE AS FILTER CARTRIDGE (DYNAMIC PERFORMANCE) HCP Depletion Performances vs residence time mAb Recovery Performances vs residence time 100% 60% 40% CHO-K1, DNA 1.000 ng/mL, HCP 120 µg/mL, IgG 2 mg/mL Dynamic conditions expose all • >75% HCP depletion can be MabXpure is designed to work with species to MabXpure's por ous achieved with 90-100% mAb classic flow rates with early data suggesting that higher flow rates structure improving the depletion of recovery. improve HCP removal. impurities. HCP Depletion Performances vs Diafiltration Buffer HCP Depletion Performances vs 2 & 3 Steps DSP platform → MM \longrightarrow MM \longrightarrow AEX 0.8 (3) (4) 4 0.8 0.4 [⊥] 0.6 **(3**) I 0.3 0.2 Post Post Post Post Post ProA MM AEX FT ProA MM ProA ProA → MM 1:25 CHO-K1, DNA 1.200 ng/mL, HCP 460-680 µg/mL, IgG 2.7-2.8 mg/mL ■MVM ■MuLV The flexibility of MabXpure allows various HCP depletion is observed after classic chromatographic steps of a diafiltration buffers from pH <6.0 to 7.5 to be used. DSP purification platform.

• With 3 chromo steps, MabXpure FT helps to streamline the process by replacing reusable flow-through technology. • For a two-step process MabXpure can allow the removal of a bind

OPTIMIZED CLARIFICATION PLATFORM

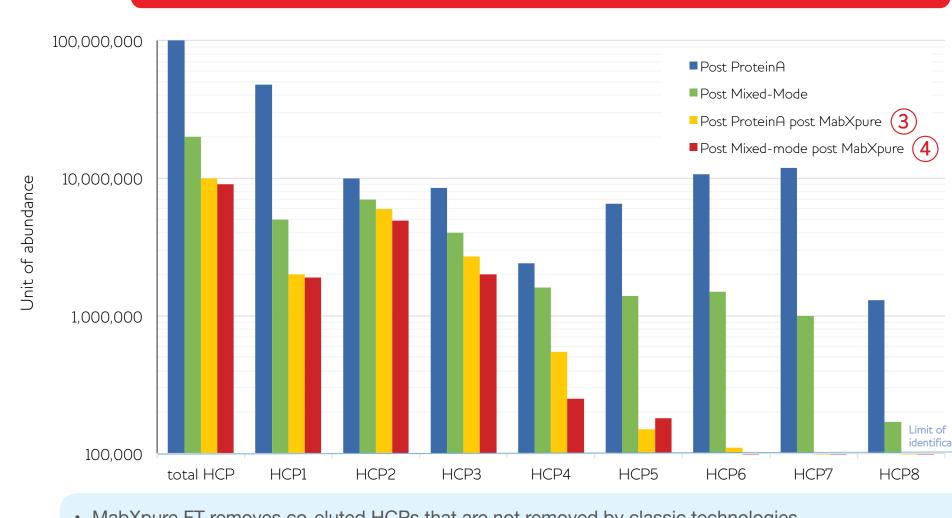


- Optimized clarification can be developed using a combination of diatomaceous earth, MabXpure, and activated carbon filled depth filter.
- Orthogonal depletion mechanisms are simultaneously taking place during the alluvial filtration step without impacting the process flow.
- After proteinA, the platform is reaching the drug substance criteria for HCP, DNA, HMW... • This combination can simplify the purification process for early tox batches, for example.

SANOFI CASE STUDY CO-ELUTED HCP DEPLETION PERFORMANCES

The highly porous structure brings high-binding

capabilities with multimodal adsorption features.



• MabXpure FT removes co-eluted HCPs that are not removed by classic technologies. • Simplified/streamlined versions of the purification platform does exhibit lower levels of co-eluted HCPs. • MabXpure FT induces purer and safer formulations for the mAbs originally containing co-eluted HCPs.

100 \$900,000 \$800,000 Time/batch (hrs) \$700,000 \$600,000 Classic MabXpure FT as Filter Cartridge \$500,000 MabXpure as Filter Aid \$400,000 MabXpure + as Filter Aid & AC \$300,000 \$200,000 \$100,000

and elute step.

COGS

- Various scenarios of MabXpure implementation are possible but the economical savings evaluation is highly linked to the process steps in place.
- Economic improvements are possible for capital expenditure and total cost per batch and gram of mAb. The use of MabXpure FT can speed-up the production time and save labor.

CONCLUSION

DAISEP MabXpure is a new, innovative, flow-through, single-use HCPs and DNA depletion technology. The use of MabXpure as a filter aid, in static mode, fits naturally within a process where depth filters are used (alluvial filtration). Ideally, MabXpure's conditions of use are optimal with high mix ratios and short contact times for sample preparation, or lower mix ratios and higher contact times for

in-process applications. In its pre-filled resin filter cartridge (RFC) design, the MabXpure FT fits very easily within a purification platform process where streamlined conditions are expected (flow-through).

MabXpure has a very high capability for depleting host cell proteins and DNA, with extremely high mAb recovery (>95%). Less intrusive to the product, it allows the removal of up to 1 LRV of HCPs, 2 LRV of DNA depending on the mode of action, and reduces co-eluted HCPs species. MabXpure can supplement, complement, or replace the pre-existing DSP unit operations.

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