



# EBA Technology: Enabling the Direct Capture of Pharma Proteins

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Second-generation Expanded Bed Adsorption (EBA) technology enables process intensification by integrating clarification and product capture into one single step; this means that centrifugation, filtration and packed bed chromatography can all be replaced by one single unit operation – resulting in less preparation and process time, and reduced investment costs.

Biopharmaceutical manufacturing of therapeutic proteins such as monoclonal antibodies (mAbs) is traditionally undertaken using mammalian cell-based production systems that excrete the full-length, matured molecule into the medium. Product recovery is a lengthy and costly procedure consisting of centrifugation and depth filtration steps to remove the cells (clarification), followed by multiple cycles of packed bed chromatography that bind and separate the target molecule from medium components (capture) (1). During this product capture step, residual host-related impurities such as DNA and host-cell proteins (HCPs) are also removed to a large extent. The current standard in IgG downstream processing is Packed Bed Protein A chromatography (using resins like MAbSelect, MabXtra and SuRe Protein A from GE Healthcare, as well as ProSepA High Cap and ProSepA Ultra High Cap Protein A media from Millipore).

In view of the high price of the ligand, cost-effective alternatives – such as cation exchange, synthetic mixed mode or single domain camel-derived antibody ligands – are also considered for antibody capture. In addition, column packing and column qualification required for the packed bed Protein A runs results in long process times and operator occupancy.

Moreover, high pressures can be observed in packed bed columns at high flow rates or in the presence of large impurities such as aggregates. In general, the Packed Bed Protein A columns need a prefiltration step to remove large particles from clarified harvest and protect the column. The fluidised bed characteristic of Expanded Bed Adsorption (EBA) using MabDirect Protein A resin eliminates this operational problem and allows process integration by combining the clarification and capture steps into one unit operation (2,3).

## THE TECHNOLOGY

Second-generation Expanded Bed Adsorption (Rhubust™) is a technology originally developed by Upfront Chromatography A/S (Copenhagen, Denmark) for protein purification from complex harvests in a very wide field (food, feed, plasma and pharma) (4). In 2010, the technology was acquired by DSM specifically for the biopharmaceutical field. As full owner of the technology, DSM is developing it further for use in house and also for external customers in the (bio) pharma field, while Upfront is still active in areas outside pharma. The use of tungsten carbide-containing, high-density agarose beads (3 g/mL) (5) in the EBA platform allows flow rates of 300 – 600 cm/hour at a typical expansion factor of two (the fluidised bed height is twice that of the resting bed), which is challenging to achieve in packed bed columns.

Once a stable bed expansion is obtained, crude, high cell-density or highly viscous and particulate feed streams can be loaded directly on the column. Cells and/or debris flow through the expanded bed unharmed while product is bound. Depending on the viscosity of the harvest or the washing solution, flow rates have to be adjusted during loading to keep the bed expansion constant. Using comparable buffers as with Packed Bed Protein A chromatography, the column is then washed and product eluted from the MabDirect Protein A under standard conditions. Resin cleaning is done using, for example, 50 mM NaOH, 1 M NaCl buffer at a contact time of 30 minutes. The MabDirect Protein A shows a similar performance to Packed Bed Protein A resins. Typical binding capacity for IgG products is 20-40 g/L resin.

The EBA technology is scalable from 1-2 cm diameter (disposable) lab-scale columns, up to 10-60 cm pilot or production column sizes (such as the Rhobust™ Flex system, see Figure 1). The different steps in the EBA process are illustrated in Figure 2.

**Figure 1:**  
Rhubust™  
Flex system





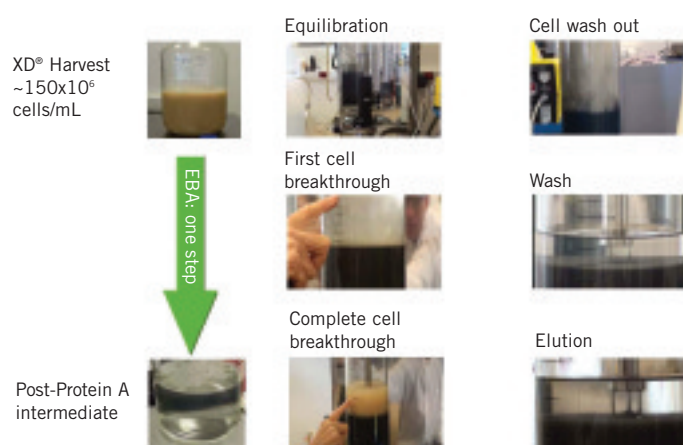
## ADVANTAGES

The main advantage of the Expanded Bed Adsorption technology is process intensification by integrating clarification and product capture into one single step. This means that the centrifugation, filtration and packed bed chromatography steps can all be replaced by one single unit operation. This results in less preparation and process time due to the reduced number of unit operations. Use of the EBA system enables direct processing of crude harvest, which eliminates the need for large centrifuges or depth filtration skids, and reduces investment costs. It is easy to operate and does not require any column packing (the resin can just be poured in). The column does not work under over-pressure like standard packed bed columns; this reduces safety risks during normal and cleaning operations. Column qualification can be done using in-process data. The EBA technology combines especially well with high titer, high cell density harvests such as DSM's proprietary XD<sup>®</sup> process and microbial processes such as *E. coli* or yeast fermentation. The XD<sup>®</sup> technology routinely delivers product titers of higher than 10 g/L and cell densities above 100 million cells/mL, which are not easily removed by depth filtration. To enable depth filtration, XD<sup>®</sup> harvests need to be diluted – which partially reduces the advantage of having high titer harvests. The EBA system copes very well with high cell density harvests, allowing efficient product capture and cell removal in one single step. Cells and debris are removed satisfactorily and eluate is sterilised by microfiltration.

## EXAMPLES

A comparison is shown in Table 1 of a traditional fed-batch process where harvest was clarified by depth filtration and product was captured by packed bed Protein A chromatography, with an XD<sup>®</sup> process where the product was directly captured from crude XD<sup>®</sup> cell culture broth using Rhobust<sup>™</sup> MabDirect Protein A. The Rhobust<sup>™</sup> technology gives higher product recoveries at similar product purity and residual impurity levels. Overall, host cell protein removal with MabDirect Protein A is the same or better compared with Packed Bed Protein A. DNA concentrations in the eluate are in the same range or slightly higher with EBA. Leached Protein A levels are in the same range, with EBA Protein A comparable with Packed Bed Protein A.

The performance of MabDirect Protein A with a CHO-XD<sup>®</sup> harvest is shown in Table 2. Samples were taken from a 50L CHO-XD<sup>®</sup> bioreactor run for the production of a monoclonal antibody, and processed in



two consecutive EBA Protein A runs. After each MabDirect Protein A run, the eluate was subjected to a low pH treatment (1 hour at pH 3.5) before neutralisation and sampling for product concentration, purity and residual impurities. The CHO-XD<sup>®</sup> harvest was successfully clarified and product captured in one single step using the EBA system without affecting product quality.

An example of a chromatogram from a MabDirect Protein A run done using the Rhobust<sup>™</sup> Flex system is shown in Figure 3A (see page 68). The MabDirect Protein A bed

**Figure 2:** EBA harvest loading through product elution

**Table 1:** Comparison of fed batch filtration and packed bed versus XD<sup>®</sup> and Rhobust<sup>™</sup>

	Recovery (%)	Purity (%)	HCP* (µg/mg mAb)	DNA (ng/mg mAb)	Protein A (ppm)
Fed-batch, filtration/Protein A packed bed	>85	>95	1-15 (n=15)	(n=2) 23-49	9-12
XD <sup>®</sup> , PrA EBA	>90	>95	0.3-23 (n=19)	3.7-121 (n=6)	4-14

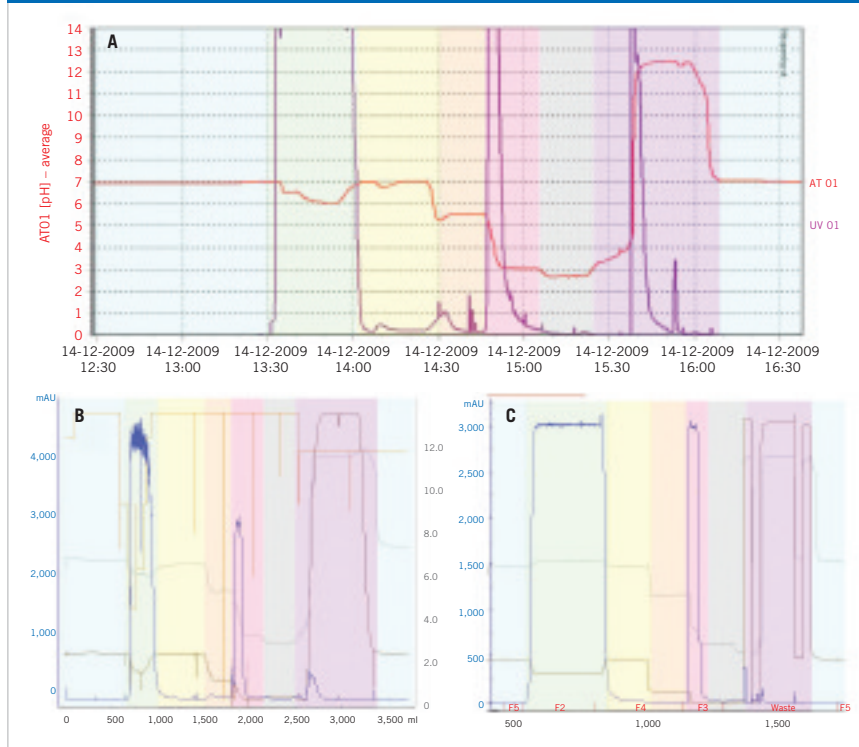
HCP = host-cell protein

**Table 2:** Rhobust<sup>™</sup> MabDirect Protein A: performance and residual impurity levels after processing CHO-XD<sup>®</sup> harvests

	EBA run	1	2
Load	Total cell density (x10 <sup>6</sup> / mL)	113	108
	Titer (g IgG/L)	8.58	9.55
Post-Protein A intermediate	Recovery (%)	94	94
	Purity (%)	96.7	96.2
	Aggregates (%)	2.3	3.8
	HCP* (µg/mg mAb)	5.31	5.40
	DNA (ng/mg mAb)	18.4	50.2

HCP = host-cell protein

**Figure 3:** Protein A chromatograms. A: Rhobust™ Flex 10 cm-diameter column MabDirect Protein A chromatogram of a Fc fusion protein. OD280 (purple) and pH (red) traces are shown. B: Rhobust™ MabDirect Protein A 2 cm diameter disposable column chromatogram of an IgG antibody. OD280 (blue), pH (grey), conductivity (brown), and flow rate (orange) traces are shown. C: Packed bed Protein A run for purification of an IgG. Colour legend: equilibration (blue), crude (A, B) or clarified (C) harvest load (green), cell wash out (yellow), pH 5.5 wash (orange), product elution (pink), strip (grey), sanitisation (lavender)



was expanded two times and equilibrated in PBS, pH 7.0 buffer. Crude harvest from a 50 L CHO-XD® run producing an Fc-fusion protein was loaded, and the cells washed through the expanded bed unharmed. After cell removal, a standard Protein A pH 5.5 intermediate wash was performed and product eluted by applying pH 3.0

elution buffer. The column was stripped and sanitised again using standard packed bed Protein A buffers. Finally the system was re-equilibrated for the next run.

Figure 3B shows a run from a 50 L CHO-XD® producing an IgG antibody, purified on a 2 cm-diameter disposable Rhobust™ column connected to an Äkta Explorer (GE Healthcare). In Figure 3C, a chromatogram of a classical packed bed protein A run is shown. Comparison of the chromatograms from EBA runs (3A and 3B) with packed bed MabSelect run (3C) shows that the elution profiles are comparable. A peak in UV signal is observed in EBA runs but is absent in packed bed experiments. The reason for this behaviour is the release of the mostly non-product related residues from the resins during stripping.

## CONCLUSION

Second-generation EBA technology presents a method for direct capture of high-value biopharmaceutical products from crude harvests, especially high cell density harvests such as XD®, thereby reducing processing time and cost without affecting product quality. It

may also be applicable to processing viscous and particulate feeds such as *E. coli* lysates or other homogenates, either as a direct capture step or as an alternative to cross-flow filtrations.

## References

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