# Eshmuno® CMX resin — a novel mixed mode cation exchange resin for the purification of glycoproteins

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## Abstract

Since the importance and number of glycoproteins has increased immensely over the last years, protein glycosylation has also gained great significance, as it influences the protein's efficacy and safety. For example, glycoproteins such as monoclonal antibodies with glycans containing high amounts of terminal mannose exhibit a faster serum clearance and decrease drug efficacy. Additionally, certain high-mannose glycans may elicit immunogenic reactions and safety issues.

Capturing and purifying highly glycosylated proteins is a difficult chromatographic task that is best approached by more than one chromatographic interaction principle.

Through combination of weak cation exchange ligands with moderately hydrophobic side chains, we have developed a novel mixed mode cation exchange resin, Eshmuno® CMX resin. The dual operation mechanism of this resin allows the purification of proteins that are difficult to separate, e.g. bispecific antibodies or antibody-drug conjugates (ADC) according to their Drug-Antibody Ratio (DAR). In addition, Eshmuno® CMX resin shows a high binding for high-mannosylated proteins, allowing the capture of those proteins as well as purification according to different glycopatterns.

## Eshmuno® CMX Resin

Eshmuno® CMX mixed mode resin is built on the proven Eshmuno® resin technology, that has unique properties in selectivity. Based on the weak cation exchange group combined with a moderate hydrophobicity, this resin enhances selectivity based on the pI and hydrophobicity of the target molecule. >90% recovery rates as well as high product binding capacity of >60 mg/mL can be achieved when using this resin.

With a broad operational window, Eshmuno® CMX resin enables use of various pH and conductivity levels to obtain high product recovery. It is also the only mixed mode resin enabling elution of hydrophobic molecules.

## Oligo-mannose glycan containing antibody separation study

An exemplary glycoprotein bind and elute separation is shown in Figure 3, displaying a part of the separation, namely the elution peak of the bound glycoprotein on Eshmuno® CMX resin at 20 mg/mL CV loading and 250 mM NaCl. Additional analytics usually are required for the collected fraction analysis.

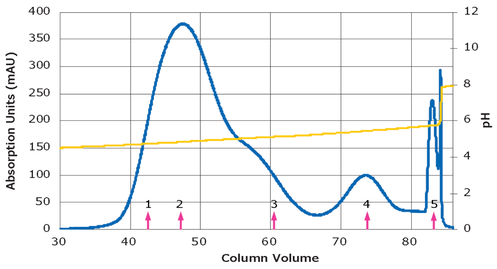


**Figure 3** – Example of pH (yellow) and UV (blue) traces detected for the chromatographic separation of an oligo-mannose containing monoclonal antibody sample loaded to 20 mg/mL CV on the mixed mode cation exchange resin. Pink marking indicates the fractions collected during the sample elution. 150 cm/h velocity was chosen throughout all the chromatographic steps.

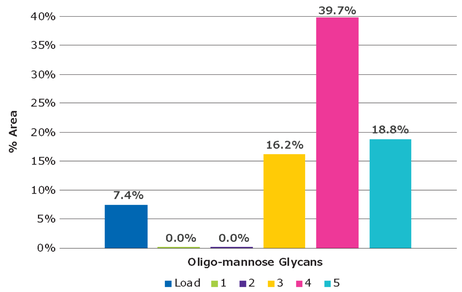


**Figure 4** – Analytical results of the sample fraction characterization using a LC-MS analytical method displayed in quantitative area of analyzed elution fractions (1 to 5), including loaded glycoprotein (load) at 20 mg glycoprotein/mL CV loading.

As shown in Figure 3 and 4, using a linear pH gradient elution the separation/enrichment of the oligo-mannose containing glycan species is possible. In the main glycoprotein containing fractions (e.g. fraction 1 and 2), no oligo-mannose variants were detected. Most of the oligo-mannose glycan species eluted in a separate fraction (e.g. fraction 4).

Alternatively, glycoprotein separation can be obtained using flow-through conditions as shown in Figure 5 and 6 where the high-mannosylated protein is bound on the column and the no oligo-mannose protein variants are in the flow-through.

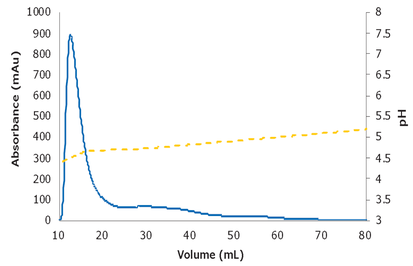


**Figure 5** – Example of pH (yellow) and UV (blue) traces detected for the chromatographic flow-through separation of oligo-mannose containing monoclonal antibody sample loaded to 10 mg/mL CV on the mixed mode cation exchange resin.

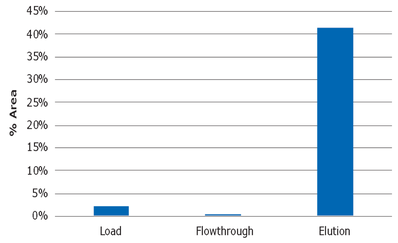


**Figure 6** – Analytical results of the sample fraction characterization using a LC-MS analytical method displayed in quantitative area of analyzed elution fractions (flow through and eluate), including loaded glycoprotein (load) at 10 mg glycoprotein/mL CV loading.

## SARS-CoV-2 Spike Protein Separation Study

The SARS-CoV-2 Spike glycoprotein consists of 674 amino acids and is extensively glycosylated with 22 N-linked glycans that are important for proper folding and modulating accessibility to host proteases and neutralizing antibodies. A high percentage of those N-glycans are oligo-mannosylated.

SARS-CoV-2 Spike glycoprotein bind and elute separation is shown in Figure 7, displaying a part of the separation, namely the elution peak of the bound glycoprotein on the separation matrix at 1 mg/mL CV loading and 250 mM NaCl.

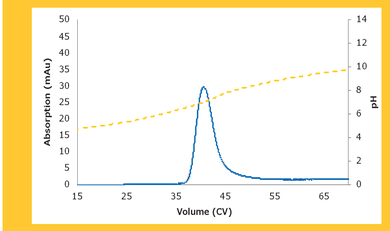


**Figure 7** – Example of pH (yellow) and UV (blue) traces detected for the chromatographic separation of oligo-mannose glycan containing protein (e.g., SARS-CoV2 Spike glycoprotein) sample loaded to 1 mg/mL CV on the mixed mode cation exchange resin.

A good binding and sharp elution of the protein during a linear pH gradient is demonstrated.

Figure 8 and 9 show analytical results of the loaded protein compared to the eluted protein on Eshmuno® CMX resin.

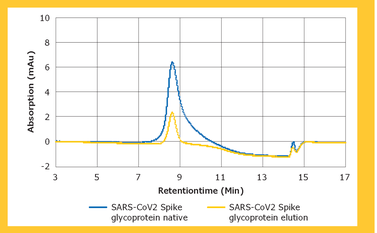


**Figure 8** – Analytical results of the sample fraction characterization using a HIC analytical method displayed in UV traces of analyzed elution fraction, including loaded glycoprotein (load) at 1 mg glycoprotein/mL CV loading.


**Figure 9** – Depicts the analytical results of fractions collected during the oligo-mannose containing protein (e.g., SARS-CoV2 Spike glycoprotein) sample separation on Eshmuno® CMX resin using SDS-PAGE as qualitative analytical method.

Both analytical methods display a further increase in purity while working with the pre-purified protein.

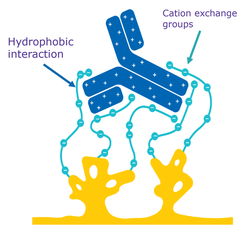


**Figure 1** - Eshmuno® tentacle structure enables more effective binding of target molecule.

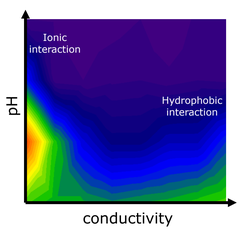


**Figure 2** - 2D plot of product static binding capacity. Red high binding to blue low binding

## Conclusion

Eshmuno® CMX resin enables the purification of glycoproteins based on the glycan pattern, increasing the protein's efficacy and safety.

The high selectivity based on the mixed mode properties facilitates the purification of SARS-CoV-2. Starting with linear screening gradients, developing and scale up of production methods is straightforward.