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Tech Note 01

Performance of 3M™ Emphaze™ AEX Hybrid Purifier in monoclonal antibody (mAb) purification process in mammalian cell cultures

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Introduction

The 3M™ Emphaze™ AEX Hybrid Purifier enables chromatographic clarification through the use of a hybrid design comprising a quaternary ammonium (Q) functional nonwoven chromatographic media paired with a size exclusion membrane. The Q-functional media is a high capacity hydrogel grafted to nonwoven. The downstream membrane is a highly asymmetric 0.2 micron nominally rated polymer membrane.

The 3M Emphaze AEX Hybrid Purifier is designed to reduce DNA, HCP, endotoxin, and cell debris. It is typically positioned as the second stage of clarification after the primary cell capture step (for example, Centrifugation or Depth Filtration). The 3M Emphaze AEX Hybrid Purifier delivers consistent, high purity clarified fluid to the capture chromatography operation. The reduction of DNA, HCP, and cell debris early in the purification process may confer benefits across the protein A column, such as higher product purity in the protein A eluate, and reduced turbidity of the neutralized viral inactivation pool.

In this tech note, we describe the performance and benefits of the 3M Emphaze AEX Hybrid Purifier in a typical monoclonal antibody purification process. We evaluate the performance in terms of turbidity reduction, HCP reduction, and DNA reduction at clarification, and its effect on protein A column purification performance.

Product Performance

Turbidity Reduction

Experimental Procedure. A non-mAb producing CHO cell centrate, having an initial turbidity of 84 NTU, a DNA concentration of 1.6×10^8 pg/mL, and an HCP concentration of 3.9×10^5 ng/mL, was passed through a 3M Emphaze AEX Hybrid Purifier BV8 capsule at a flux of 190 L/(m^2 h). Samples for turbidity, DNA, and HCP analysis were taken periodically throughout each run. In addition, the final pool samples were taken. The outlet turbidity is shown in Figure 1, the DNA reduction in Figure 2, and the HCP reduction in Figure 3.

Dynamic Binding Capacity for DNA

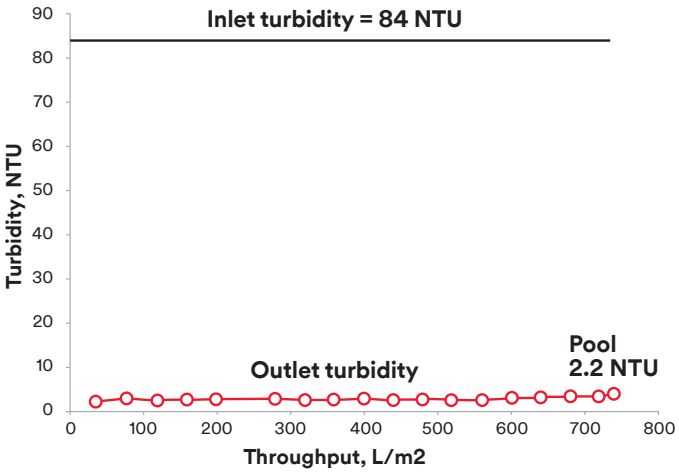


Figure 1. *Outlet turbidity as a function of throughput for 3M™ Emphaze™ AEX Hybrid Purifier*

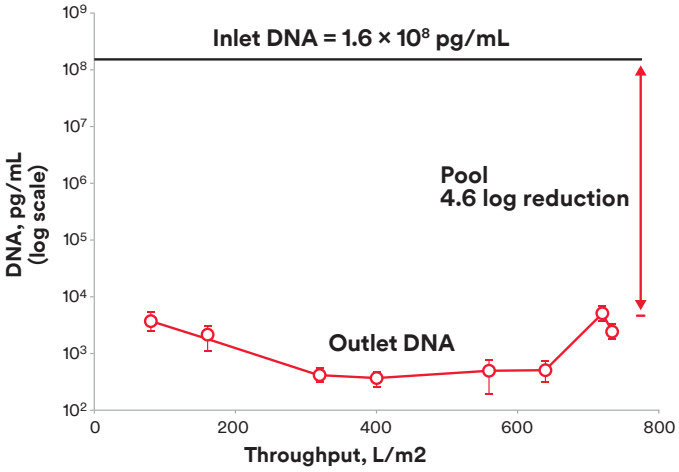


Figure 2. *DNA Reduction as a function of throughput for 3M™ Emphaze™ AEX Hybrid Purifier.*

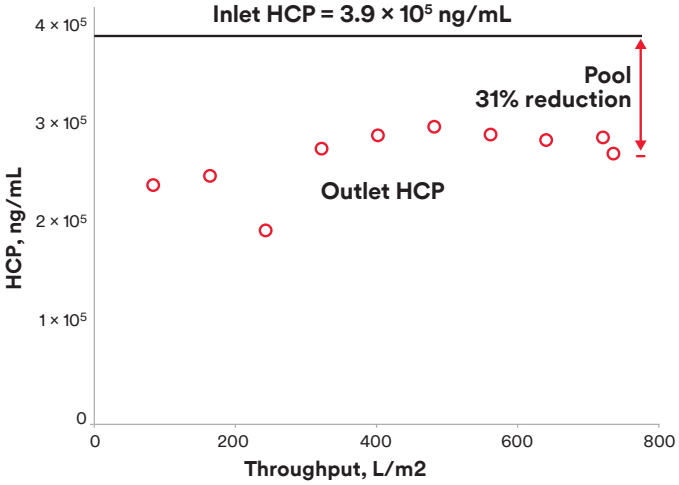


Figure 3. *HCP Reduction as a function of throughput for 3M™ Emphaze™ AEX Hybrid Purifier.*

The DNA Dynamic Binding Capacity (DBC) of the 3M™ Emphaze™ AEX Hybrid Purifier using pure challenges is shown in Figure 4 as a function of buffer type, and in Figure 5 as a function of pH and conductivity. Figure 5 shows a trend of increasing DNA DBC with increasing NaCl concentration in these pure challenges. (Note: The data presented are for information purpose only based on performance of product meeting all release criteria and should not be regarded as product specification. Results using low conductivity solutions (1.5 mS/cm) are for comparison only and are outside the recommended use conductivity range.)

Experimental Procedure. Reference solutions with a known concentration of Calf Thymus DNA were prepared in the appropriate loading buffer. The DBC test was performed using an automated liquid chromatography system that continuously monitors the UV absorbance. The DBC was determined at 10% breakthrough relative to the UV absorbance of the DNA challenge solution. The samples were flushed with equilibration buffer at 5 BV/min until a constant UV baseline was achieved. Equilibration buffer had the same composition of the reference solution but contained no DNA. The 3M Emphaze AEX Hybrid Purifier media composite was then challenged with the DNA solution at 5 BV/min.

Product Recovery

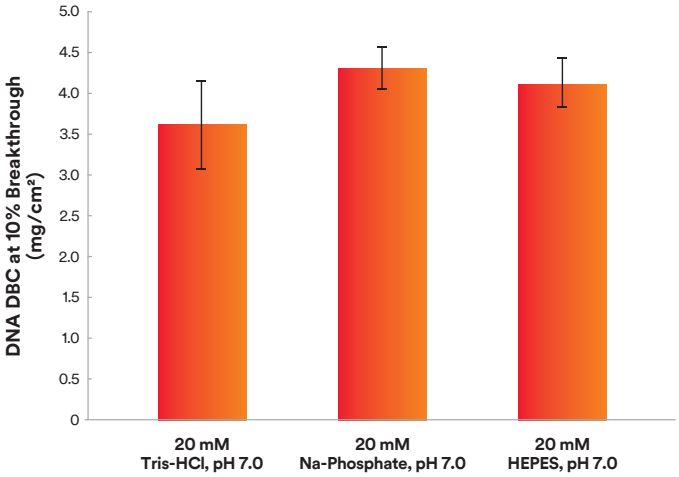


Figure 4. *DNA Dynamic Binding Capacity (DBC) of 3M™ Emphaze™ AEX Hybrid Purifier as a function of buffer type. No additional sodium chloride was introduced during buffer preparation.*

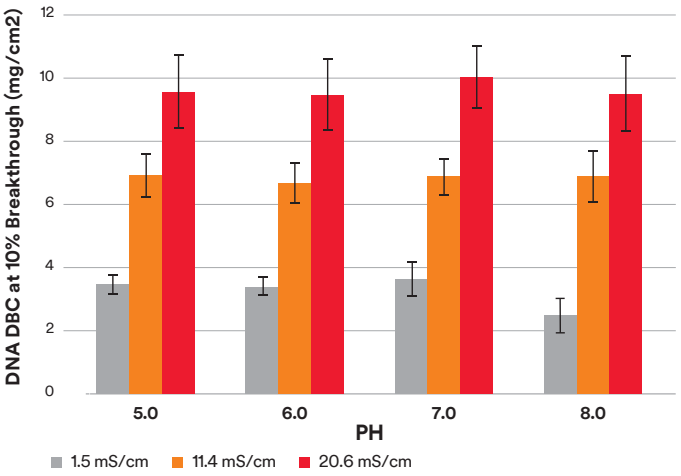


Figure 5. *DNA Dynamic Binding Capacity (DBC) of 3M™ Emphaze™ AEX Hybrid Purifier as a function of pH and conductivity. Note: The data presented are for information purpose only based on performance of product meeting all release criteria and should not be regarded as product specification. Results using low conductivity solutions (1.5 mS/cm) are for comparison only and are outside the recommended use conductivity range.*

The 3M™ Emphaze™ AEX Hybrid Purifier primarily uses an anion exchange mechanism for purification. This results in low nonspecific binding of the cationic product and consequently mAb recoveries > 95% can be attained.

Experimental Procedure. Human IgG recovery was used as a model of mAb recovery by the 3M Emphaze AEX Hybrid Purifier. A human immunoglobulin G (IgG) challenge solution was prepared by dissolving 1.0 mg/mL of IgG and 100 mM of NaCl in 20 mM phosphate buffer, pH 7. A 3M Emphaze AEX Hybrid Purifier capsule was challenged with 380 L/m^2 of this challenge solution at a flux of 3.8 L/(m^2-min). The resulting IgG recovery was 99.5%.

Enhanced Protein A Column Performance

The purification performance of the protein A chromatography column may be enhanced by significantly reducing DNA and HCP load using 3M Emphaze AEX Hybrid Purifier in the upstream clarification process.

HCP reduction was evaluated in Experimental Procedure A discussed below. The HCP concentration was reduced by 24% when the centrate was processed through a 3M Emphaze AEX Hybrid Purifier. This is compared to only a 6% reduction with a 3M depth filter. This higher level of clarified fluid purity resulted in an HCP concentration in the protein A eluate 19 times lower for the 3M Emphaze AEX Hybrid Purifier case compared with the 3M depth filter case.

Experimental Procedure A. A non-mAb producing CHO centrate having a turbidity of 84 NTU was spiked with 0.7 mg/mL human IgG (as a mAb simulant). The spiked centrate was clarified using either a 3M depth filter or 3M Emphaze AEX Hybrid Purifier, then loaded onto a protein A column that was subsequently washed and eluted. An aggressive clean-in-place (CIP) procedure was performed on the protein A column using 4 M guanidine HCl. HCP was quantified, using ELISA, at each of the numbered positions 1-4 in Figure 7 (post-centrifugation, post-clarification, post-elution from protein A, and in the fluid recovered from the protein A column during the CIP procedure).

DNA reduction was evaluated in Experimental Procedure B. Figure 8 shows the DNA concentration measured at each numbered step in the process sequence for cases in which the

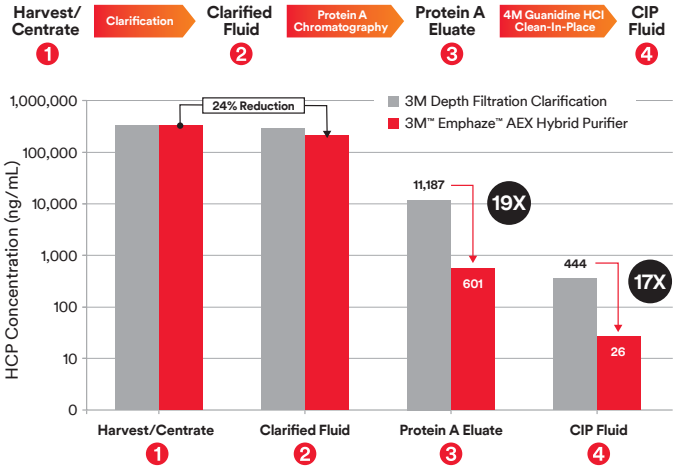


Figure 6. *HCP concentrations in CHO harvest or centrate (1), clarified fluid (2), protein A eluate (3), and clean-in-place fluid (4) when CHO harvest/centrate was clarified using either 3M Depth Filtration clarification (grey) or 3M™ Emphaze™ AEX Hybrid Purifier (red).*

clarified fluid was spiked with IgG. Whereas this experiment indicated no measurable reduction of DNA by the 3M depth filter, 3M Emphaze AEX Hybrid Purifier reduced the DNA concentration in the CHO centrate by 3.9 LRV. This resulted in a cumulative DNA reduction of 5.9 LRV post-protein A (a 3.5-log improvement in cumulative post-protein A DNA reduction compared with 3M depth filtration). These results indicate substantially better performance of the protein A column in clearing DNA when the 3M Emphaze AEX Hybrid Purifier was used for upstream clarification, compared with the 3M depth filter. In fact, the 3M Emphaze AEX Hybrid Purifier clarified fluid had a 1.5-log lower DNA concentration than the protein A eluate for the 3M depth filter.

DNA/protein complexes such as chromatin have been reported to facilitate co-elution of HCP with mAb from the protein A column.[1,2] These reports are consistent with the

findings herein, showing that >4 log removal of DNA during clarification prior to protein A chromatography can result in a many-fold reduction in HCP impurities in the protein A pool.

Experimental Procedure B. A non-mAb producing CHO centrate was clarified using either a 3M depth filter or 3M™ Emphaze™ AEX Hybrid Purifier. The clarified fluid was then spiked with 1.0 mg/mL human IgG as a simulant for mAb. The IgG-spiked fluid was then loaded onto a protein A column that was subsequently washed and eluted. An acid regeneration of the protein A column was performed using 5 column volumes of citric acid buffer, pH 2.5. DNA was quantified by qPCR at each of the numbered positions 1-4 in Figure 8 (post-centrifugation, post-clarification, post-elution from protein A, and in the fluid recovered from the protein A column during acid regeneration).

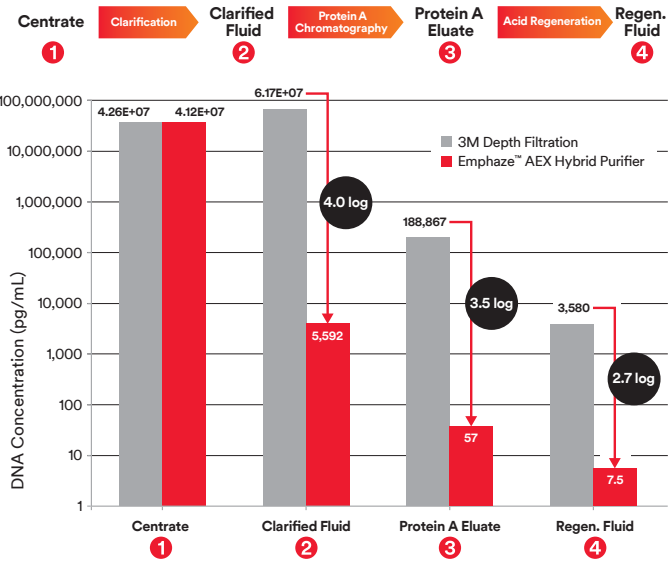


Figure 7. *DNA concentrations in CHO centrate (1), clarified fluid (2), protein A eluate (3), and acid regeneration fluid (4) when CHO centrate was clarified using either a 3M depth filter (grey) or 3M™ Emphaze™ AEX Hybrid Purifier (red).*

Enhanced protein A column protection

The 3M Emphaze AEX Hybrid Purifier provides protection of the valuable capture chromatography column by decreasing the contaminant load, resulting in measurably fewer column-bound impurities. This has the potential to enable less aggressive column cleaning procedures.

The data provided in Figure 6 demonstrates that using a 3M Emphaze AEX Hybrid Purifier for clarification results in a substantial reduction of column-bound protein impurities as evidenced by the low HCP concentration in the regeneration fluid (CIP fluid). In this study the HCP concentration in the CIP fluid was only 26 ng/mL, which was 17 times lower than that resulting from a 3M depth filter clarified load.

Similarly, as shown in Figure 7, the DNA concentration in the regeneration fluid was 2.7 logs lower for a 3M Emphaze AEX Hybrid Purifier clarified load compared to 3M depth filter clarified fluid, indicating substantially reduced column-bound DNA impurities after elution.

Summary

The 3M Emphaze AEX Hybrid Purifier is a ***"hybrid clarifier,"*** a synthetic, clarifying product containing both a Q-functional anion exchange media and a fine particle reduction membrane.

- **Robust turbidity reduction.** The 3M Emphaze AEX Hybrid Purifier provides consistent outlet turbidity < 5 NTU throughout the entirety of the clarification run, due to the high anion exchange capacity.
- **Substantial DNA reduction.** The 3M Emphaze AEX Hybrid Purifier provides greater than 4 log DNA reduction.
- **Excellent mAb recovery.** The 3M Emphaze AEX Hybrid Purifier's chemically defined, cationic media has a very low nonspecific binding which provides excellent mAb recovery, typically >95%.
- **Enhanced performance of the protein A column.** The 3M Emphaze AEX Hybrid Purifier, when used in the upstream clarification stage, can substantially enhance purification performance of the protein A chromatography column by significantly reducing DNA and HCP challenge load.
- **Enhanced protection of the protein A column.** The 3M Emphaze AEX Hybrid Purifier provides protection of the valuable capture chromatography column, resulting in substantially fewer column-bound impurities. This has the potential to enable less aggressive column cleaning procedures.

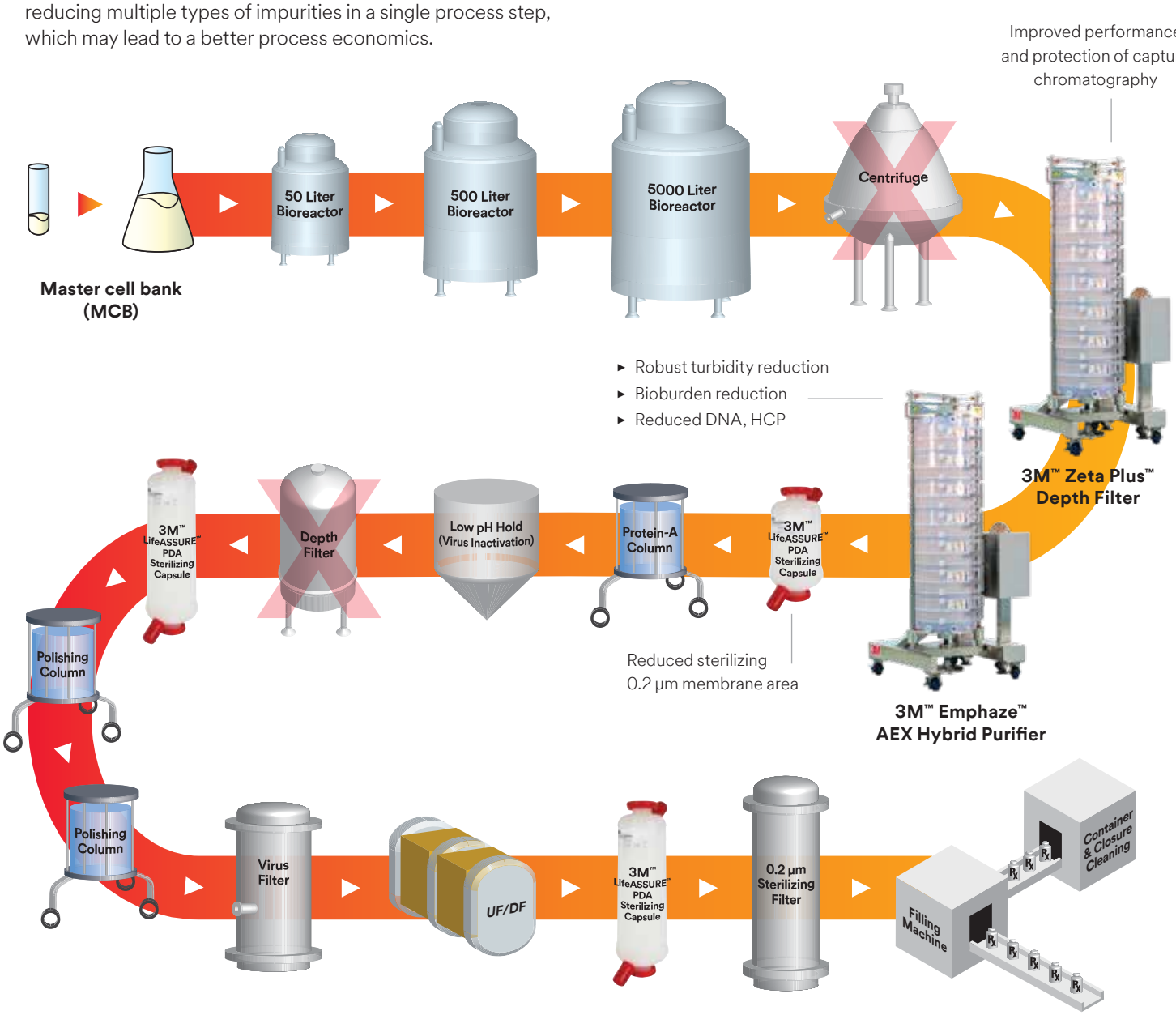
Conclusion

The 3M™ Emphaze™ AEX Hybrid Purifier brings chromatographic clarification to the bioprocess. Specifically, this product is intended for use in the clarification of mammalian cell cultures as part of the purification of biopharmaceuticals, particularly monoclonal antibodies (mAbs). The 3M Emphaze AEX Hybrid Purifier enables chromatographic clarification through the use of a hybrid media design comprising a Q-functional nonwoven chromatographic media paired with a size exclusion membrane. This enables simultaneous reduction of cell debris, DNA, HCP, and endotoxins. Reduction of key contaminants early in the process may simplify customers' processes by reducing multiple types of impurities in a single process step, which may lead to a better process economics.

References

1. H. T. Gan, J. Lee, S. M. A. Latiff, C. Chuah, P. Toh, W. Y. Lee & P. Gagnon; Characterization and removal of aggregates formed by nonspecific interaction of IgM monoclonal antibodies with chromatin catabolites during cell culture production; *Journal of Chromatography A* **2013**, 1291, 33-40.

2. P. Gagnon, F. Hensel, S. Lee & S. Zaidi; Chromatographic behavior of IgM:DNA complexes; *Journal of Chromatography A* **2011**, 1218, 2405-2412.



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