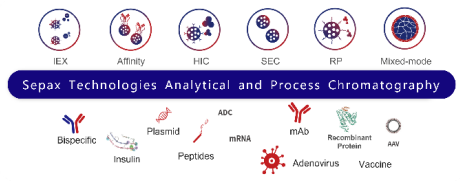
Sepax Technologies, Inc.



Bispecific Antibody Purification with Sepax MabPurix Protein A Affinity Chromatography

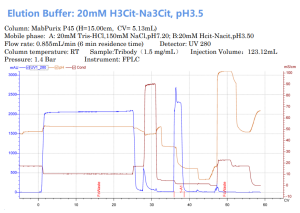
Bispecific antibodies (bsAbs) are designed antibodies with two binding sites for two different epitopes on two types of antigens or on the same antigen based on the knowledge of each of the two individual monoclonal antibodies (mAbs). This is made possible by utilizing various design platforms for creating bsAbs combining protein engineering and recombinant DNA technologies, along with effective and sustainable downstream purification and manufacturing processes. There are two categories of bsAbs: IgG-like and non-IgG like. IgG-like bsAbs bear two Fab arms and one Fc region, where mismatching and mispairing events generates nine out of ten possible combinations that are to be removed by downstream purification process development; while non-IgG like bsAbs which lack an Fc region include various strategies of linking two Fabs or scFvs together through chemically or genetically created linkages. In each case, selecting the right resin and associated purification scheme plays significant roles in the development of any bsAb before they can progress in the pipeline.

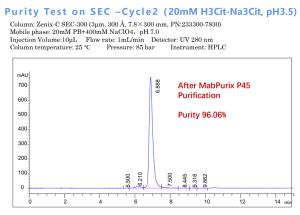
In this newsletter, Sepax Technologies, Inc. showcases the use of MabPurix P45 Protein A Affinity Resin as the capture step for the purification of two bispecific antibody samples, including one IgG-like Tribody (with Fc region) and another non-IgG *Kappa* light chain bsAb.

The initial process development for both cases was challenging, due to the low expression level of the target in the crude sample. Sepax MabPurix P45 was able to successfully provide high purity and yield with low HCP and Protein A leaching levels. BsAb purity was analyzed by SEC-HPLC with Sepax Zenix-C SEC-300 analytical column. Titer and DBC determination were performed with Sepax ProAqa Excel Protein A affinity column.

Application: Tribody Purification - MabPurix P45

For bsAbs with Fc region, Protein A affinity resins effectively capture such molecules including all the mismatches which must be separated from the desired bispecific pair by resorting to IEX or HIC resins.





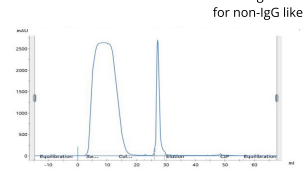
In this study, three different elution buffers were screened in the capture step of this Tribody purification. Over 95% purity was achieved in all three conditions while the method with 20mM H3Cit-Na3Cit, pH3.5 provided the highest purity (96.06%), lowest HCP residual (78.58 ppm) and Protein A leaching level (16.96 ppm).

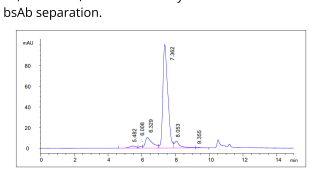
Resin	Cycle	Elution	Column Volume [mL]	DBC Capacity [mg/mL]	Elution Volume [mL]	Tribody Concentration in Eluent [mg/mL]	HPLC-SEC Purity [%]	HCP Residual [ppm]	Protein A Leaching [ppm]
MabPurix P45	1	50mM HAC-NaAC, pH3.5	5.13	36	10.52	19.76	95.55	107.89	19.26
	2	**20mM H3Cit-Na3Cit, pH3.5**			13.65	15.24	**96.06**	**78.58**	**16.96**
	3	100mM Gly-HCl, pH3.5			10.16	19.68	95.57	112.80	32.52

Click here for full application KM1002

Application: Bispecific Antibody Purification - MabPurix P45

For bsAbs lacking Fc domains, Fab regions are targets of affinity binding. Protein L is known to specifically bind to Kappa light chain, however, Protein L affinity ligand has the shortcomings such as low dynamic binding capacity, unstable in 0.5 M NaOH that is required for effective CIP, and an expected elution pH of 2.5 which is undesirable. On the other hand, Protein A is shown to bind to a β strand of the VH domain without interacting with CDR, therefore, Protein A affinity resins can be utilized for non-IgG like bsAb separation.

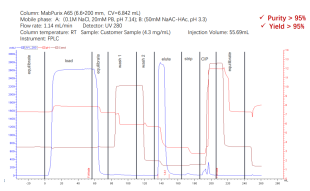




For this challenging sample, the unique selectivity of the Sepax MabPurix P45 enabled a superior performance when screened against other Protein A and L brands: with ~80% purity and over 40 mg/mL of DBC were achieved with the lowest ppm level of HCP and Protein A leaching in the evaluation. Sepax Monomix Ion Exchange resins can be used to further purify and improve the yield as the next step in the process development.

Click here for full application KM1001

Application: Monoclonal Antibody Purification - MabPurix A65



Sepax Technologies also offers agarose-based Protein A affinity resins, MabPurix A65

- High DBC: 50 mg hIgG/mL at 5 min short residence time
- Stability up to 0.5 M NaOH

> 95% purity and > 95% yield were achieved after the MabPurix A65 capture step, in this monoclonal antibody showcase.

Click here for full application KM1004

Technical Specifications of Sepax MabPurix Protein A vs. Other Brands

Click here for more information: KM1003

Product Info

Product	Particle Size	Part Number	Pack Size (L)	Cartridge (mL)
MabPurix P45	45 µm	270845990	0.5, 1, 5, 10, 100	4.2
MabPurix A45	45 µm	270745990	0.5, 1, 5, 10, 100	4.2
MabPurix A65	65 µm	270765990	0.5, 1, 5, 10, 100	4.2

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