Sepax Custom Affinity Chromatography Showcase:

Custom Affinity Using HSA as the Immobilized Ligand to Assess mAb Immunogen Cross-Reactivity

Samples: ERBITUX® (cetuximab), RITUXAN® (rituximab), and Vectibix® (panitumumab)

HSA - An Immobilized Ligand

Human Serum Albumin (HSA) is an abundant protein in the human body synthesized by the liver, and is highly conserved among many mammalian species (especially domain 1). With a molecular weight (MW) of approximately 66.5 kDa, HSA has many cysteine AA sites, which makes it capable of binding many ligands (especially lipid-type molecules).

Albumin also transports many endogenous molecules including hormones, fatty acids, metabolites, and other exogenous compounds including drugs, it also buffers pH and maintains oncotic pressure.[1] Therefore, HSA can greatly influence drug half-life, which is critical to pharmacokinetics and efficacy.

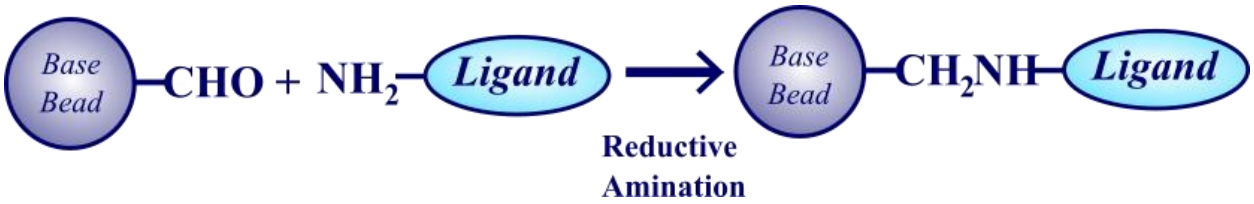
Because of HSA's critical role in drug transport, it imperative and necessary to assess potential drug interactions during development of modern therapeutics, such as Monoclonal Antibodies (mAbs).

A well-controlled method to assess such immune cross-reactivity in vitro would be to immobilize HSA to a specific biocompatible resin matrix and evaluate the degree of interaction of HSA with a target mAb of interest.

Optimization of Immobilization

Sepax has optimized a method to immobilize HSA using a surface modified polystyrene support with free aldehyde reactive sites. The aldehyde easily reacts to the amine group of the lysine amino acid in the HSA. The aldehyde reaction with the primary amine forms an imine also known as a Schiff base (C=N) with the release of water. In the presence of sodium cyanoborohydride, the Schiff base is reduced to highly stable C-N bond.

Figure 1 *below illustrates the reaction between the activated support and the amine from the HSA or other ligand of choice.*

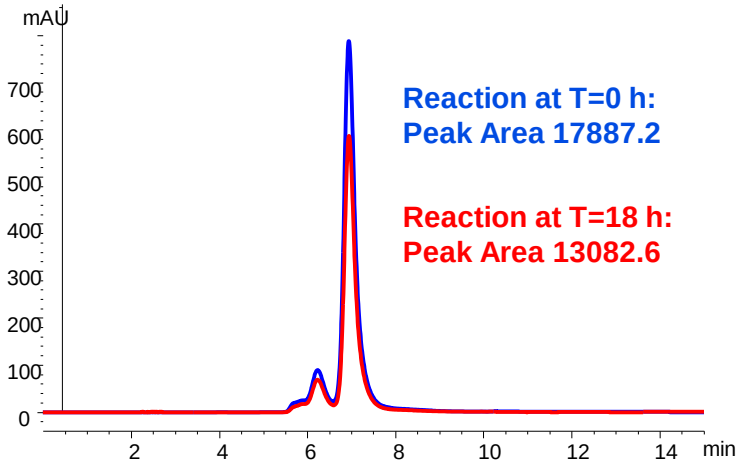


Monitoring HSA Coupling Reaction

A Sepax Zenix 150A SEC column was used to measure free HSA (in blue) from time point 0 and time point 18hrs (red). The peak areas correspond to how much HSA is bound to the resin.

It monitors the reaction rate with respect to time for the surface modification process for Sepax Bioservice Affinity HSA.

The chromatogram shows that 27% of the HSA was bound, as peak absorbance decreased proportionately



Column to Monitor Reaction: HPLC Column, Sepax, Zenix SEC-150, 3um, 150 A 4.6 x 300 mm (P/N: 213150-4630); Mobile phase: 150 mM sodium phosphate, pH 7.0; Isocratic:15 min; Flow rate: 0.35 mL/min; Detector: 280 nm; Column temperature: 25 °C; Injection 5 µL

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Immobilized Ligands Assessing HSA-mAb Immunogen Cross-Reactivity

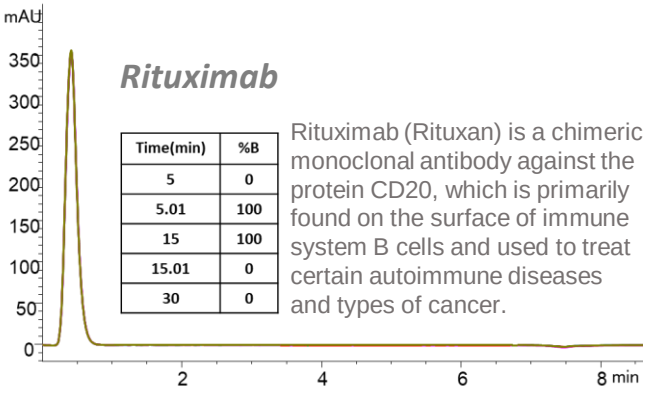
The specific immobilization chemistry utilized on the activated resin matrix, such as: epoxy, oxidation/reduction, reductive amination, and glutaraldehyde, to name a few, is critical in determining the conjugation efficiency of the ligand of choice (HSA) and its elution profile. The cross-reactivity of a mAb with immobilized HSA may provide critical insight regarding mAb half-life, likelihood for adverse immune reactivity, and its potential efficacy.

Such immobilization techniques can be used to assess other critical interactions between a drug (small or large molecule) and the target in vivo.

For example, immobilization of the key cytochrome proteins (such as cytochrome C, P450, and other variants) can give valuable insights on how a small molecule is metabolized. Such techniques serve as an effective and well control technique to predict toxicity and half-life for a broad class of individuals without having to rely on murine models. For larger molecules, various proteins such as IgG1, transferrin, IgM, ceruloplasmin, and etc.) can be immobilized to assess cross-reactivity as well.

Immobilization of these components can be assessed in a fixed bed system in series or mixed bed format in various ratios that mimic endogenous environment. The goal of such immobilizations allows for improved pre-clinical models for variants that exists in diverse human populations minimizing adverse reactions or more accurately predicting contraindications.

MAbs on Sepax BioService Affinity HSA Analytical Column



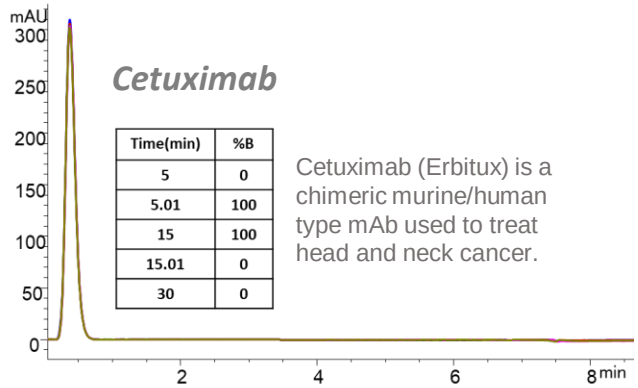
Rituximab

Time(min)	%B
5	0
5.01	100
15	100
15.01	0
30	0

Rituximab (Rituxan) is a chimeric monoclonal antibody against the protein CD20, which is primarily found on the surface of immune system B cells and used to treat certain autoimmune diseases and types of cancer.

The profiles below show 20 µg injections of mAbs (Cetuximab, Rituxmab, or Pantumummab) eluting in the flow through at RT 0.5 min and no absorbance at 7.5 min.

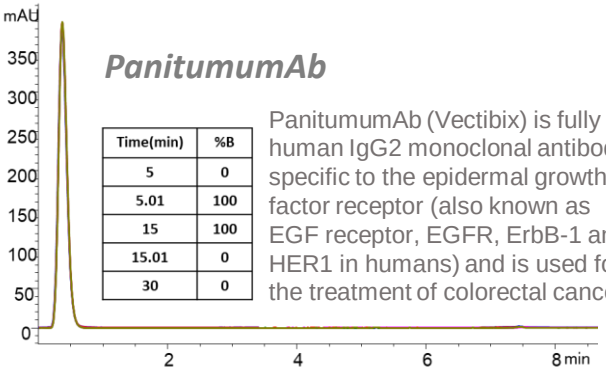
This suggests no binding to HSA indicating lack of cross-reactivity.



Cetuximab

Time(min)	%B
5	0
5.01	100
15	100
15.01	0
30	0

Cetuximab (Erbitux) is a chimeric murine/human type mAb used to treat head and neck cancer.



PanitumumAb

Time(min)	%B
5	0
5.01	100
15	100
15.01	0
30	0

PanitumumAb (Vectibix) is fully human IgG2 monoclonal antibody specific to the epidermal growth factor receptor (also known as EGF receptor, EGFR, ErbB-1 and HER1 in humans) and is used for the treatment of colorectal cancer.

Column : Sepax BioService Affinity HSA 2.1 x 50 mm PEEK (P/N: 272101P-2105); Mobile phase A: 20mM phosphate,150mM NaCl, pH 7.5; B: 0.1 M Glycine, pH 2.5; Flow rate: 0.5 mL/min; Detector: 280 nm; Column temperature: 25 ° C; Sample: 2 mg/mL; Injection Amount: 10 µL

Showcase:

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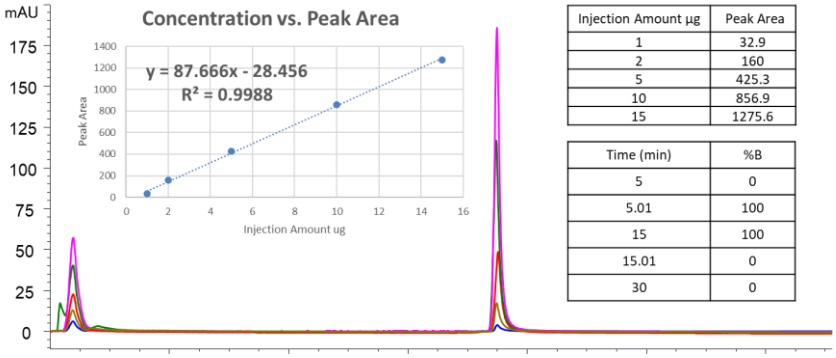
Sepax BioService Affinity HSA Analytical Column

Linearity Assessment (Sample: Anti-HSA 1 mg/mL)



Injection Amount µg	Peak Area
1	32.9
2	160
5	425.3
10	856.9
15	1275.6

Time (min)	%B
5	0
5.01	100
15	100
15.01	0
30	0

Column : Sepax BioService Affinity HSA 2.1 x 50 mm PEEK (P/N: 272101P-2105); Mobile phase A: 20mM phosphate,150mM NaCl, pH 7.5; B: 0.1 M Glycine, pH 2.5; Flow rate: 0.5 mL/min; Detector: 280 nm; Column temperature: 25 ° C

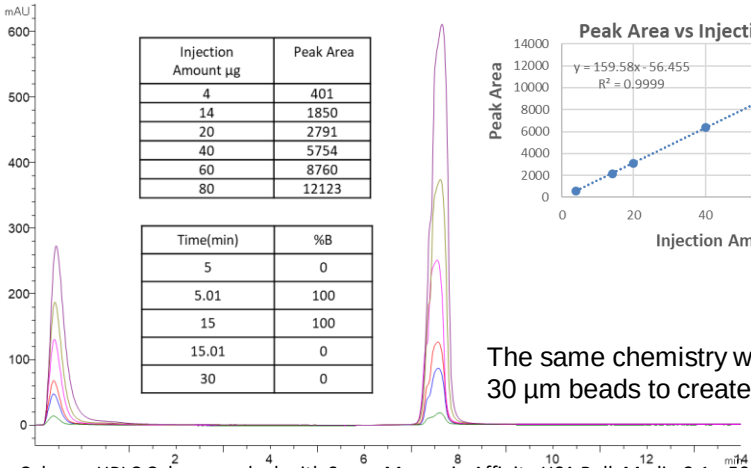
The resin was then washed and packed in a 2.1 x 50mm column and equilibrated with phosphate buffer containing NaCl. Linearity was assessed using anti-HSA ranging from 1-15 µg with mobile phase B 0.1 M glycine, pH 2.5.

The profile reveals an R2 coefficient of 0.999 indicating a linear range from 1-15 µg. The peaks at ~7.5 min have a very "sharp" elution profile with minimal tailing.

This provides evidence that there is minimal non-specific binding. The peaks around 0.5 min represent the flow through from the sample matrix of the anti-HSA.

Sepax Monomix Affinity HSA Bulk Resin – Scale up

Scalability Assessment (Sample: Anti-HSA 2 mg/mL)



Injection Amount µg	Peak Area
4	401
14	1850
20	2791
40	5754
60	8760
80	12123

Time(min)	%B
5	0
5.01	100
15	100
15.01	0
30	0

The results shows linearity up to 80 µg with an LOD of less than 0.02 µg.

The broad linear range demonstrates that our 30 µm beads may be suitable for higher loading needs of large-scale purification experiments.

The same chemistry was used in effort to demonstrate scalability using 30 µm beads to create Sepax Monomix HSA Affinity Bulk Media.

Column: HPLC Column packed with Sepax Monomix Affinity HSA Bulk Media 2.1 x 50 mm PEEK (P/N: 272201P-2105); Mobile phase A: 20mM phosphate,150mM NaCl, pH 7.5; B: 0.1 M Glycine, pH 2.5; Flow rate: 0.4 mL/min. 0-5 min 100%A, 5.1 to 15 min 100%B, 15.1 to 35 min 100%A; Detector: 280 nm; Column temperature: 25 ° C

Conclusion

HSA can be immobilized effectively on our biocompatible activated supports and that cross-reactivity can be assessed on various mAbs. Anti-HSA elution profile comparison with commercially available mAb elution profiles demonstrate target specific interaction with minimal cross-reactivity.

Scale up experiments with 30 µm beads confirm achievable technology transfer from analytical commercial scale. It is also important to note that the techniques used in this study may also be used to immobilize other clinically significant markers for predicting possible cross-reactivity, mAb half-life, and toxicity of small/large molecules.