Affinity Services

Sepax Technologies has been working with a variety of customers for over the last decade supporting multiple projects that involve resin and ligand chemistry selection for the best possible affinity performance. Some of the immobilization chemistries include, epoxy, NHS, Streptavidin Biotin, reductive amination, and disulfide exchange. We have successfully immobilized many ligands using our advanced chemistries and surface modifications.

As resin chemistry experts, we come equipped with over 12 polymer chemists with extensive experience in resin and ligand design. This coupled with our experience separates us from the rest. We have been successful in many applications and continue to expand our technical horizons.

Key Areas:

- Fusion Proteins
- Cross Reactivity
- RNA purification
- Glycosylated compound capture
- mAb Purification
- BsMabs Purification

Working in tandem with our scientists and our customers, we offer highly individualized services to meet your specific requirements, helping you reach project goals in an efficient and cost-effective manner.

In addition to these services, Sepax also provides **OEM** and **ODM** services.

Ligand Examples

DNA Immobilization – for microarrays and sensors

Heparin – protein purification, cation exchanger, and DNA purification

Lectin – purification of glycoconjugates/ glycans/ glycoproteins

Boronate – simple ligand selectivity for glycated species

RNA – any dT (10,20,30 etc) useful for purification for RNA constructs

mAb/anti-mAb – specific interaction with various proteins

Protein A – purification of mAb's and some bsmAb's

Enzyme – clinical diagnostics, lab Assays, bio-catalysis

IgM –myoglobin analysis and other reactive species

IgG/HSA – cross interaction chromatography for drug ½ life studies and competition against other antigens

Heterodimer Affinity – separations of bispecifics in fermentation broths for 2-D analysis with SEC or in process monitoring

Custom Resins – immobilization of small molecule/protein for drug interaction/metabolism

Let one of our **subject matter expert** today to walk you through the process of our methodology for creating affinity media from start to finish.

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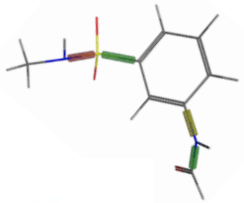
Resin Matrix Type, Immobilization Chemistry, and Corresponding Particle Size

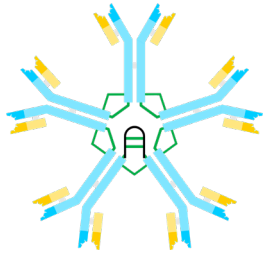
Ligand Type	Chemistry	Resin Matrix	Particle Size (µm)
➢ IgM ➢ Mab ➢ DNA ➢ RNA ➢ HSA ➢ Enzyme ➢ Boronate ➢ Protein A ➢ Heterodimer ➢ Heparin ➢ Custom Ligand	➢ Extended Chain NHS ➢ Streptavidin-Biotin (noncovalent attachment) ➢ Reductive Amination (reactive aldehyde) ➢ Extended Chain Epoxy ➢ Extended Chain CNBr ➢ Coupling Method of Customer's Choice	➢ Agarose ➢ PS-DVB ➢ Silica ➢ PMA ➢ Surface Modified PMA ➢ Surface Modified PS-DVB ➢ Surface Modified Silica (case by case basis) ➢ Customer Provided Matrix	➢ Silica: 5, 10, 20, 40, 60 ➢ Agarose: 45, 65, 90 ➢ PS-DVB: 5, 10, 20, 30, 60 ➢ PMA: 10, 30, 60

Ligand Types

Custom Ligand



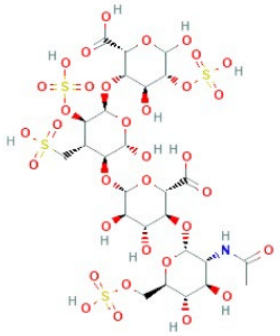
IgM

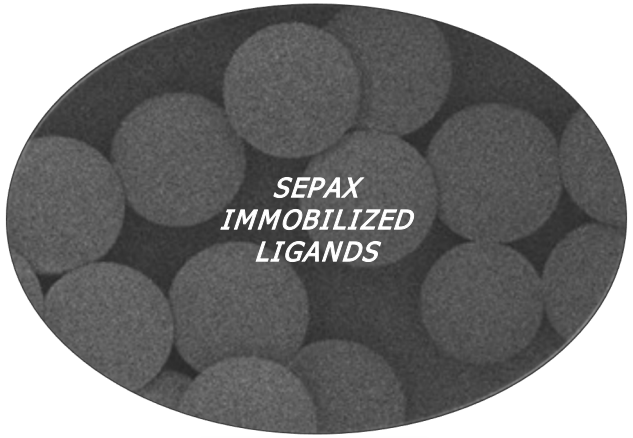


mAb

DNA

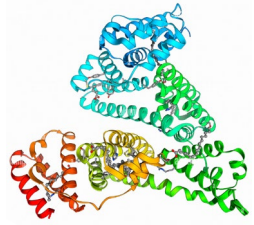
Heparin



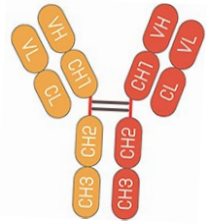


RNA

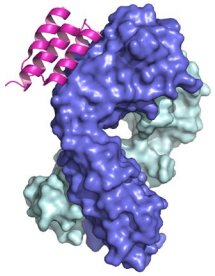
HSA



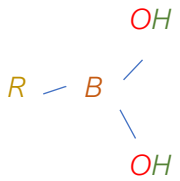
Heterodimer



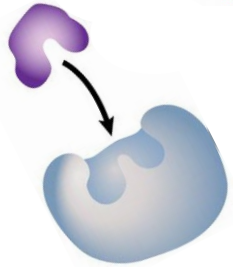
Protein A



Boronate



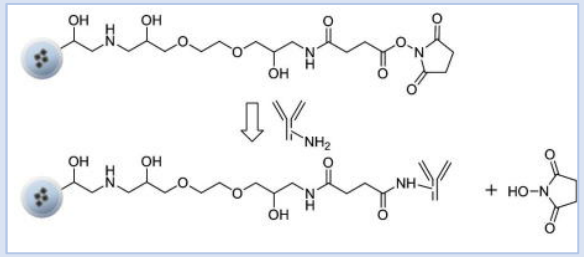
Enzyme



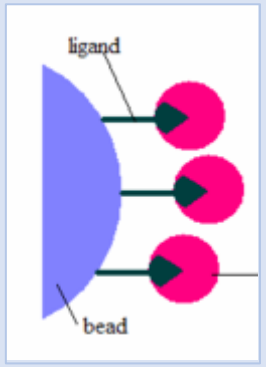
Affinity Process

1. Resin Selection

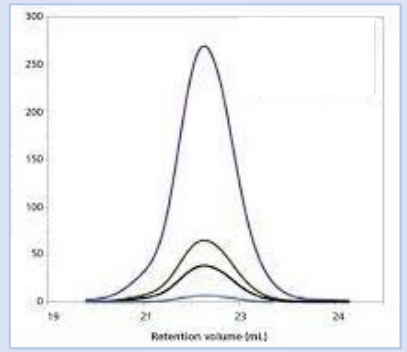
2. Determine Ligand/ Conjugation Type and Amount



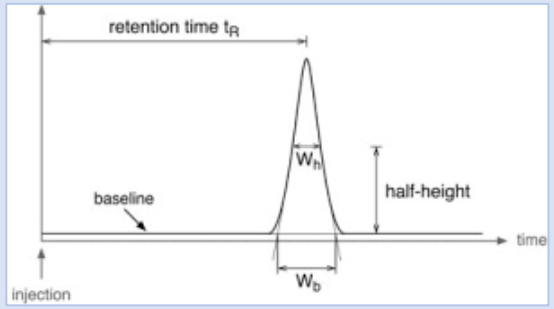
3. Immobilization



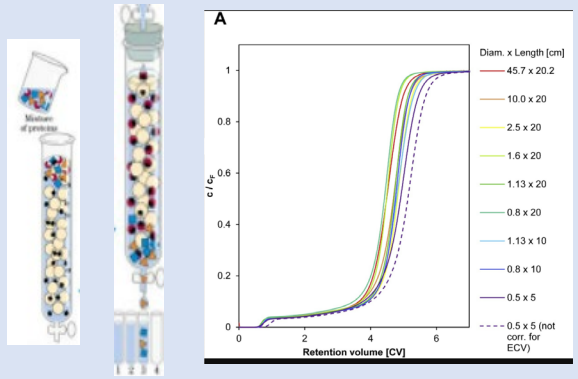
4. Conjugation Efficiency by SEC



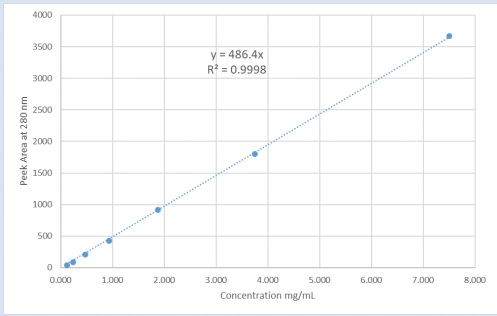
5. Column Packing and Plate Count Measurement



6. DBC Testing



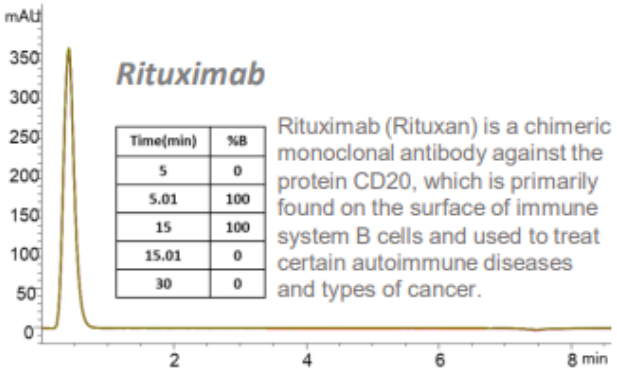
7. Linearity/LOD/LOQ Qualification



Immobilization Study - HSA

HSA is a ligand used to 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. interaction with drugs including mAbs and can reveal stability (increased presence in blood stream) but also can limit bioavailability if bound to tight.

mAbs on Sepax BioService Affinity HSA Analytical Column

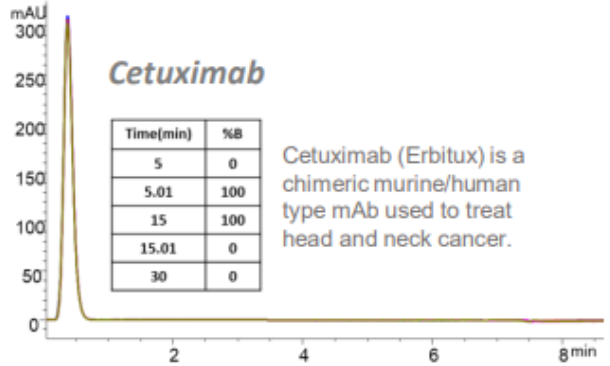


Time(min)	%B
5	0
5.01	100
15	100
15.01	0
30	0

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.

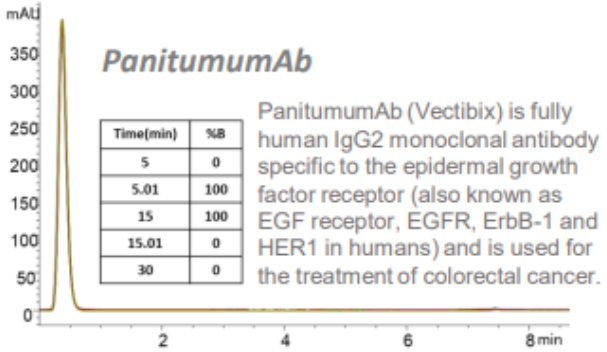
The profile below shows 20 ug injections of mAbs (Cetuximab, Rituxmab, or pantuymmab) eluting in the flow through at RT 0.5 min and no absorbance at 7.5 min.

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



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

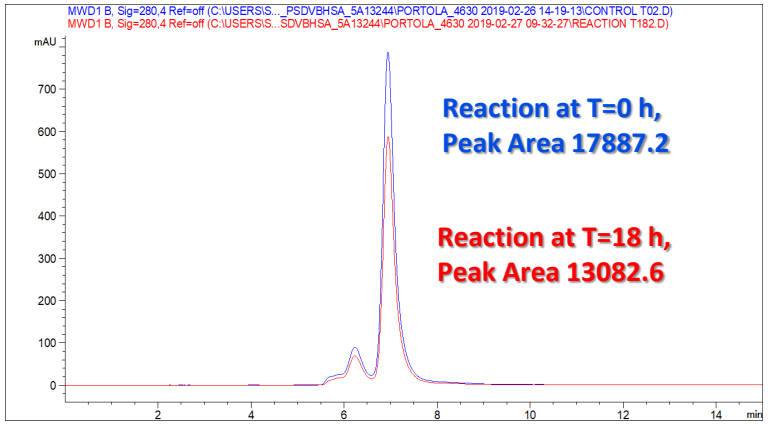
Cetuximab (Erbitux) is a chimeric murine/human type mAb used to treat head and neck cancer.



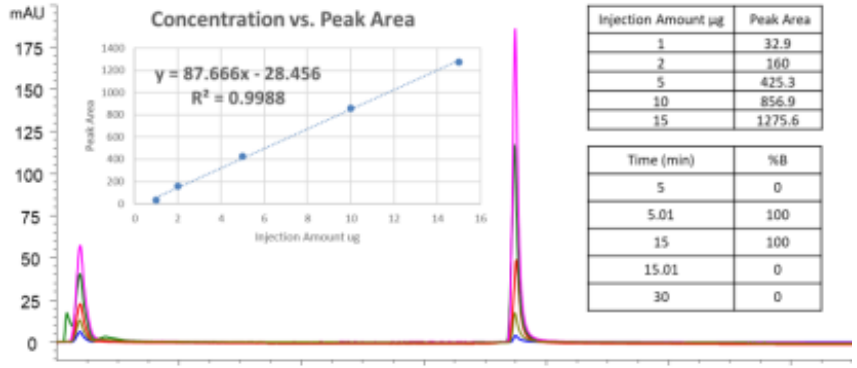
Time(min)	%B
5	0
5.01	100
15	100
15.01	0
30	0

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



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

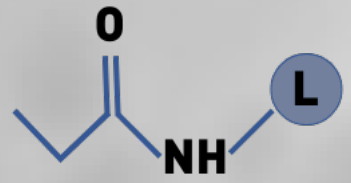


Injection Amount µg	Peak Area
1	32.9
2	160
5	425.3
10	856.9
15	1275.6

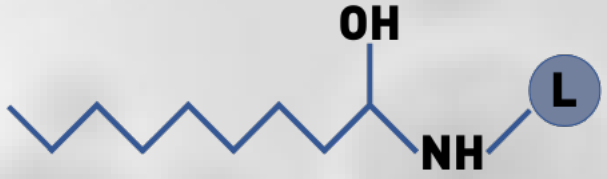
Time (min)	%B
5	0
5.01	100
15	100
15.01	0
30	0

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

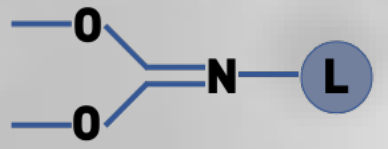
Disulfide Exchange



NHS Coupling



Epoxy Ring Coupling



CNBR/NaOH

Reductive Amination

Contact Us

Sepax has developed an in-process method that measures the accuracy, conjugation efficiency, and reliability of ligand binding. Resin selection screening and linker chemistry reduces nonspecific interaction and optimizes loading capacity. The covalent attachment of ligand to the linker bound to the matrix coupled with size exclusion chromatography quantitatively measures the binding efficiency. This allows for the optimal conjugation time and the highest possible ligand loading. From there, column packing efficiency is measured along with dynamic binding capacity (continuous feed to 10% breakthrough). Once capacity and packing is optimized, accuracy measurements are performed assessing linearity, LOD, LOQ, and spiked sample accuracy.

Contact Us

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Sepax-tech.com/Affinity_Services.php

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