MabSelect™ VL resin

AFFINITY CHROMATOGRAPHY

MabSelect™ VL affinity resin (Fig 1) uses a protein L ligand with strong affinity for the variable region of a human antibody's kappa light chain. The resin offers high productivity and robust processes for affinity capture of bispecific antibodies and antibody fragments containing the kappa light chain and offers a good capture alternative for antibody variants that does not bind to protein A. This resin has substantially improved dynamic binding capacity (DBC) and alkaline stability compared to its predecessor, making it well suited for cost-efficient capture of antibody variants. MabSelect™ VL resin allows for good resolution of product-related impurities in the capture of bispecific antibodies, and it provides a tool for efficient purification of antibody variants to high purity.

Key features of MabSelect™ VL resin:

- High binding capacity for bispecific antibodies and antibody fragments containing a kappa light chain.
- Stable when cleaned with 0.1 M NaOH, reducing risk for bioburden incidents.
- Provides good resolution for product-related impurities in the capture of bispecific antibodies.

Antibodies are the largest class of biotherapeutics. As this class grows, so does its diversity — projects in research through to commercial manufacturing increasingly involve variants such as bispecifics, conjugates, or fragments. Platform approaches have eased the development of purification protocols for many monoclonal antibodies (mAbs) on the market, but selecting a purification scheme for antibody variants can be challenging, given the wide range in the pipeline. Protein L interacts with the kappa light chain and allows for capture of bispecific antibodies and antibody fragments as well as removal of mispaired species, providing an alternative for purification of antibody variants when protein A is not suitable or sufficient. The main characteristics of MabSelect™ VL resin are summarized in Table 1.

Fig 1. MabSelect™ VL resin is available in bulk and in prepacked columns.

Table 1. Main characteristics of MabSelect™ VL resin

Matrix	Highly cross-linked agarose, spherical
Ligand	Alkaline stabilized protein L-derived (*E. coli*)
Ligand coupling	Single point attachment
Coupling chemistry	Epoxy
Particle size d_{50v} [1]	~ 60 µm
DBC Q_{B10} [2]	~ 70 mg human IgG/mL resin at 6 min residence time ~ 60 mg human IgG /mL resin at 4 min residence time
Recommended maximum operating flow velocity	300 cm/h [3]
pH stability, operational [4]	2 to 10
pH stability, CIP [5]	2 to 13 (recommended 0.1 M NaOH)
Chemical stability	Stable in aqueous buffers commonly used in protein L chromatography.
Delivery conditions	20% ethanol or 2% benzyl alcohol (BnOH)

[1] Median particle size of the cumulative volume distribution
[2] DBC at 10% breakthrough by frontal analysis at a mobile phase velocity of 100 cm/h (6 min residence time) and 150 cm/h (4 min residence time) in a lab column at 10 cm bed height for human IgG in PBS buffer, pH 7.4
[3] Packed in an AxiChrom™ 300 column with 30 cm i.d. at 20 cm bed height, using buffers with the same viscosity as water at 20°C
[4] pH range where resin can be operated without significant change in function
[5] pH range where resin can be subjected to cleaning-in-place (CIP) without significant change in function

Designed for high productivity and robustness

The increased diversity of antibody variants drives the need for chromatography resins that bind different sites of the antibody molecule (Fig 2). Protein A capture resins use the interaction of protein A with the fragment crystallizable (Fc) region and sometimes with the variable region of the antigen-binding fragment (Fab) heavy chain (VH3). As a complement to protein A, protein L can be used to bind molecules containing kappa variants of the variable light (VL) chain.

Protein L binds human kappa light chain subtypes 1, 3, and 4. Table 2 summarizes the binding profile of protein L.

In biomanufacturing, the need for cost-efficient and robust processes drives demand for high-capacity, alkaline-stable resins. Whereas Capto™ L resins were designed to be cleaned with 15 mM NaOH, the protein L ligand in MabSelect™ VL resin can withstand 0.1 M NaOH. This improved alkaline stability is due to modification of one domain from the native protein L ligand, together with multimerization (Fig 3).

Binding capacity and alkaline stability

In a study of binding capacity, we observed a two-fold increase in binding capacity of MabSelect™ VL resin compared to its predecessor, Capto™ L resin (Fig 4). This higher capacity provides productivity advantages in biomanufacturing. MabSelect™ VL also provides high alkaline stability, as observed in an accelerated alkaline stability study (Fig 5) and a CIP cycling study (Fig 6). Accelerated studies provides quicker results, whereas CIP cycling studies represents how resins are cleaned in the industry. In the accelerated alkaline stability study, the column was exposed to 0.1 M NaOH for 4 h, which corresponds to 16 CIP cycles of 15 min each. The incubation was repeated multiple times. The dynamic binding capacity was measured with trastuzumab, and the relative remaining capacity was calculated between incubations. After 80 CIP cycles, the relative remaining DBC for MabSelect™ VL resin was 100%, whereas the relative remaining DBC of Capto™ L resin was 60% (Fig 5). In the CIP cycling study (Fig 6), we measured relative remaining DBC of MabSelect™ VL resin over 175 CIP cycles using buffer and 0.1 M NaOH. Relative remaining DBC for trastuzumab was unchanged up to 150 cycles then dropped slightly. Note that this study was done with buffer; a larger loss in DBC can be expected when cycling with cell supernatant, as protease activity and possible fouling of the resin may affect purification outcomes. Greater alkaline stability enables a more robust process and longer resin lifetime, adding to overall process economy.

Table 2. Protein L antibody binding affinities (1)

Species	Antibody class	Affinity*
General	Kappa light chain (subtypes 1,3, 4)	Strong
	Lambda light chain	No binding
	Heavy chain	No binding
	Fab	Strong
	ScFv	Strong
	Dab	Strong
Human	IgG1	Strong
	IgG2	Strong
	IgG3	Strong
	IgG4	Strong
	IgA	Strong
	IgD	Strong
	IgE	Strong
	IgM	Strong
Mouse	IgG1	Strong
	IgG2a	Strong
	IgG2b	Strong
	IgG3	Strong
	IgM	Strong
Rat	IgG1	Strong
	IgG2a	Strong
	IgG2b	Strong
	IgG2c	Strong
Pig	Total IgG	Strong
Dog	Total IgG	Weak
Cow	IgG1	No binding
	IgG2	No binding
Goat	IgG1	No binding
	IgG2	No binding
Sheep	IgG1	No binding
	IgG2	No binding
Chicken	Total IgG	No binding

* Binding affinities apply only to species and subtypes that contain appropriate kappa light chain.

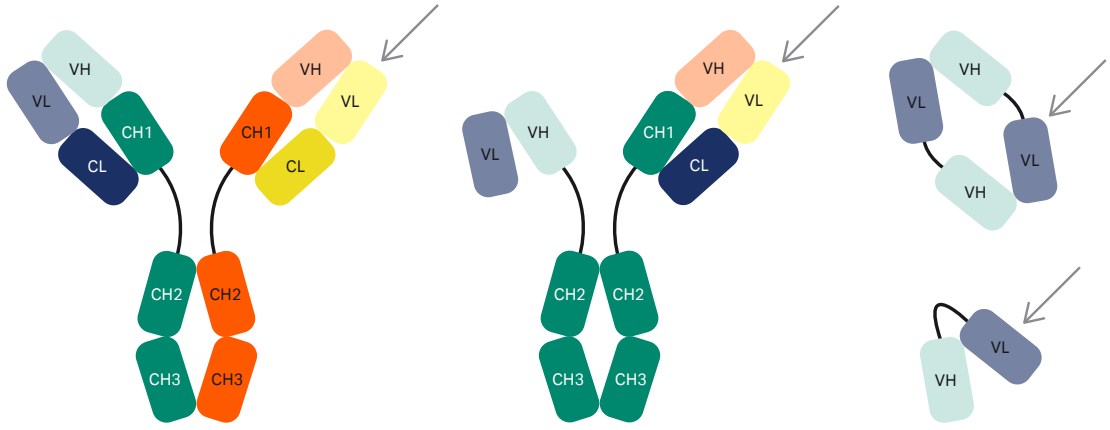

Fig 2. Antibody variant molecules. The variable light chain (VL), indicated with arrows, can have kappa or lambda variants. The protein L ligand on MabSelect™ VL resin has affinity for three of four kappa light chain variants and allows for capture of bispecific antibodies and antibody fragments.

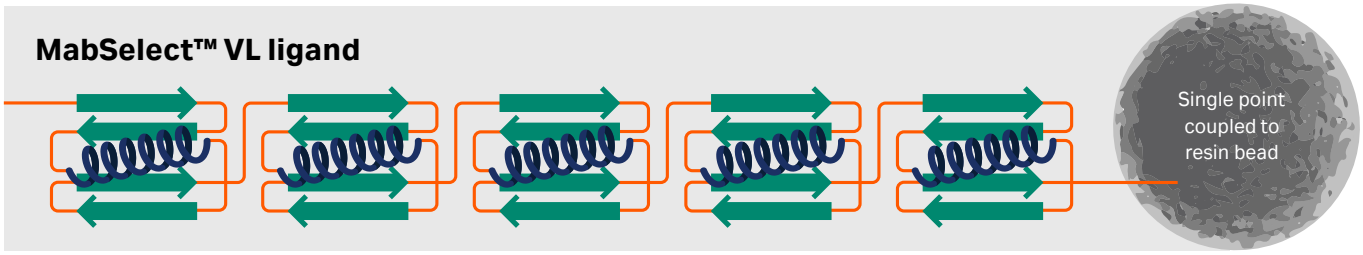

Fig 3. One domain of native protein L ligand has been genetically engineered and multimerized for better alkaline resistance on the resin bead.

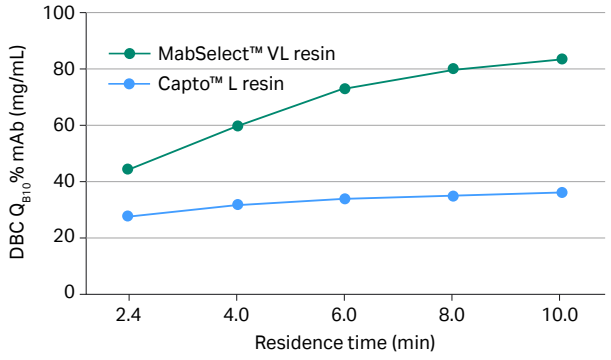

Fig 4. DBC at 10% breakthrough (Q_{B10}) vs residence time (RT) of MabSelect™ VL resin and the predecessor Capto™ L resin.

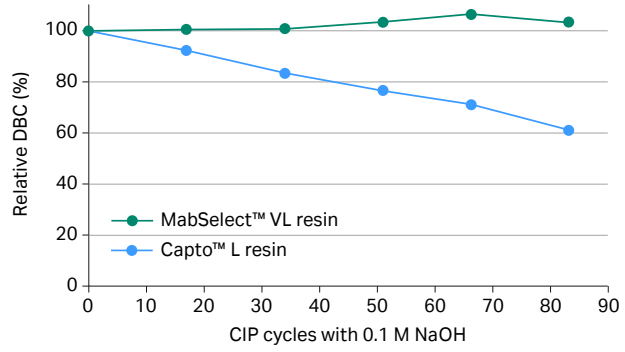

Fig 5. Accelerated alkaline-stability study of relative remaining DBC for MabSelect™ VL and Capto™ L resins over 80 cleaning-in-place (CIP) cycles with 0.1 M NaOH.

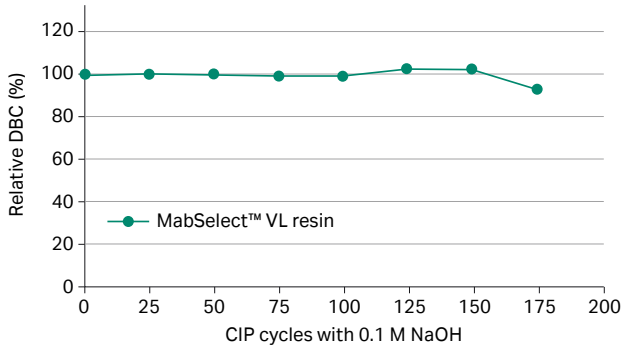

Fig 6. CIP cycling study of relative remaining DBC to test alkaline stability for MabSelect™ VL resin over 175 CIP cycles with 0.1 M NaOH.

Determining ligand leakage

We have developed a protocol for determining the MabSelect™ VL resin ligand leakage by ELISA in herceptin mAb eluate.

After purification using a standard protocol, determine yield of the mAb purification step spectrophotometrically, adjust eluate to a neutral pH, and heat treat the sample for 60 min at 95°C. Develop a standard curve for the MabSelect™ VL protein L ligand, diluted in the relevant mAb at the same concentration as the eluate, and heat treat in parallel with the eluate.

Assay the supernatant of the eluate and quantitate against the standard curve for MabSelect™ VL protein L. We used a commercially available protein L ELISA kit (sold and manufactured by Medicago AB) and follow the manufacturer's instructions, except for the final detection antibody incubation with substrate, which we did for 30 min instead of the recommended 10 min. For further information or to request free protein L ligand, please contact your Cytiva sales representative.

Base matrix properties

The base matrix allows for a range of residence times and thus is suitable for many different process conditions and objectives. The rigid bead can be used with linear flow rates up to 300 cm/h. The base matrix is also used for the MabSelect PrismA™ protein A resin and is suitable for GMP manufacturing processes, as its rigid pressure/flow properties remain constant through to large-scale columns.

Capture of bispecific antibodies and removal of product-related impurities

Purification of antibody variants brings additional challenges compared to working with conventional mAbs. Product-related impurities due to mispairing and half antibodies add complexity to the purification process. Removal of product-related impurities at the capture step can help simplify subsequent polishing steps.

MabSelect™ VL resin provides good resolution of product-related impurities. Figure 7 shows a chromatogram for capture of a bispecific antibody using MabSelect™ VL resin. The mispaired lambda homodimer does not bind to the column, as it does not contain the kappa light chain, and can be found in the flowthrough. The gradient elution from pH 5.5 to pH 2.5 separated the mispaired kappa homodimer and the kappa-lambda heterodimer bispecific and thereby removed the product-related impurities.

Column: Tricorn™ 5/100 packed with MabSelect™ VL resin
Sample: 50 mL bispecific mAb feed, 0.1 mg/mL (30% kappa light chain, 30% lambda light chain, 40% Fc)
Elution buffer: 50 mM citrate

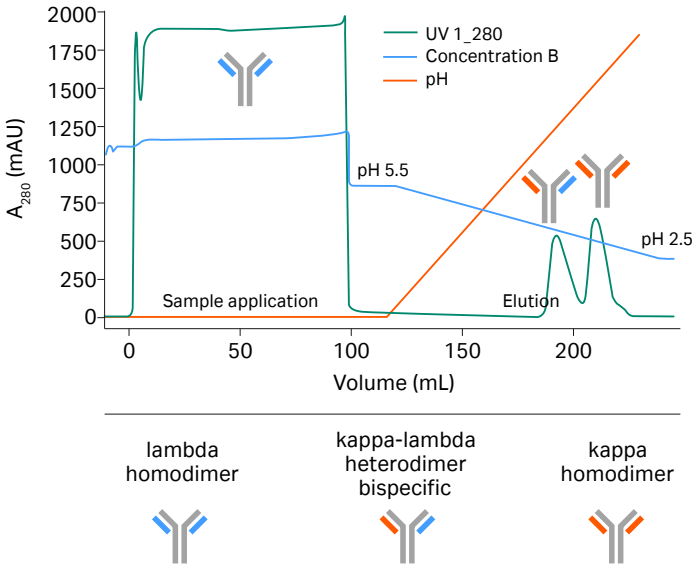

Fig 7. Chromatogram of separation of a kappa-lambda heterodimer bispecific antibody from product-related impurities.

Formats for research through to commercial manufacturing

MabSelect™ VL resin is available in 25 mL samples and in HiTrap™ 1 mL columns. Contact your Cytiva sales representative for more information.

The following formats for different scales and purposes will be offered in the future. In addition to prepacked 1 mL HiTrap™ columns, we will offer 5 mL HiTrap™ columns and 25 mL and 200 mL packs of bulk resins, which are well suited for research. For process development workflows, MabSelect™ VL resins will be available in PreDictor™ plates, RoboColumn™ units, prepacked HiTrap™ and HiScreen™ columns, and in bulk for packing in Tricorn™, HiScale™, or AxiChrom™ chromatography columns. When scaling up to clinical and commercial scale, larger containers (1 L, 5 L, and 10 L) will also be available. These will be shipped in 20% ethanol or in 2% benzyl alcohol (BnOH) upon request. We will also offer ready-to-use ReadyToProcess™ columns in a range of sizes (1 L, 2.5 L, 5 L, 10 L, 20 L, 32 L, and 57 L). These single-use columns enable fast setup, reduce cross-contamination risk, and flexibility for quick adjustment of production scales.

A capture chromatography toolbox for antibody variants

Affinity chromatography separates proteins on the basis of a reversible interaction between the target protein and a specific ligand attached to a chromatography base matrix. As diversity in the pipeline of therapeutic antibodies expands, so does toolbox for capturing antibody variants. Figure 8 provides a guide for selecting an affinity chromatography resin based on target antibody variant molecule.

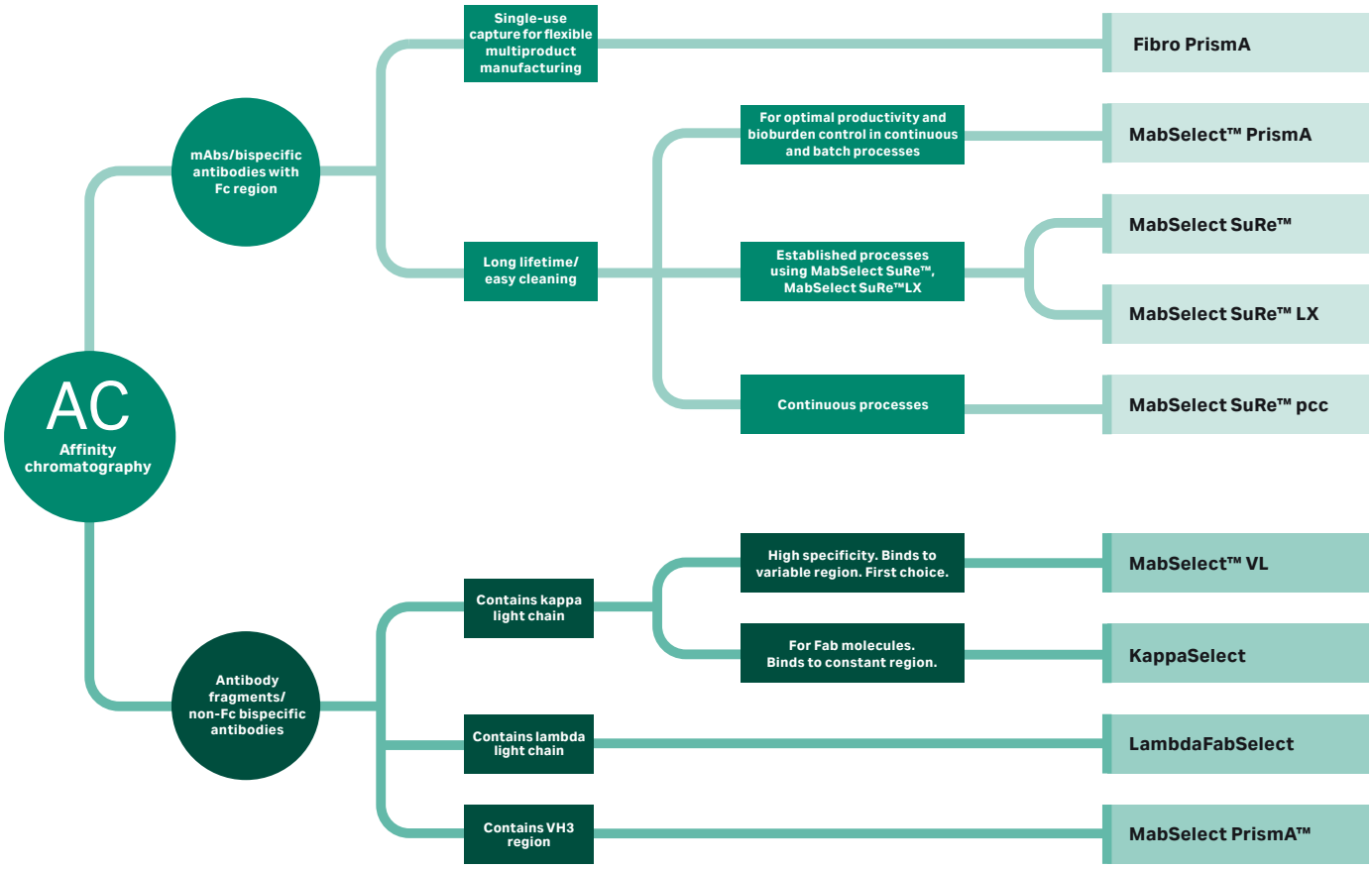

Fig 8. Selection tree for affinity chromatography (AC) resins for purification of antibody variants.

Supply chain stability

The complex nature of biopharmaceuticals makes manufacturing a challenge, in which delivering a consistent, high-quality end product is dependent on the use of equally consistent, high-quality manufacturing components. Cytiva continues to make significant investments in capacity expansion and supply stability to ensure reliable and consistent supply of our chromatography resins and prepacked ReadyToProcess™ columns. We recommend customers work closely with our commercial teams to forecast demand to support our production planning and manufacturing operations.

For emergency preparedness, we have made significant investments and implemented efforts to minimize the risk and impact of any potential supply interruptions in our manufacturing. Cytiva's chromatography product manufacturing has been certified to ISO22301 Business Continuity Management standards. As an extra precaution in the event of any disruption of our supply chain, we've created a strategic reserve of chromatography resins used in approved manufacturing processes, to support ongoing supply coverage during the recovery phase. Resin types, volumes, and storage locations of the reserve are regularly reviewed to ensure effective deployment of materials globally should an incident occur.

Support and training

MabSelect™ VL resin belongs to the BioProcess™ family of products developed and supported for large-scale manufacture of biopharmaceuticals. This support includes validated manufacturing methods, secure long-term resin supply, and regulatory support files (RSF) to assist process validation and submission to regulatory authorities. In addition, Fast Trak™ training and education provide high-level, hands-on training in key aspects of process development and manufacturing.

Resin storage

Store unused MabSelect™ VL resin in its container at a temperature of 2˚C to 8˚C. Ensure that the screw top is fully tightened. Equilibrate packed columns in buffer containing 20% ethanol or 2% BnOH. After storage, equilibrate with starting buffer and perform a blank run, including CIP, before use.

Ordering information

Product	Size	Product code
HiTrap™ MabSelect™ VL column	1 × 1 mL	17542051
HiTrap™ MabSelect™ VL column	5 × 1 mL	17542052
Products to be released in 2022		
MabSelect™ VL resin	25 mL	To be determined
MabSelect™ VL resin	200 mL	To be determined
MabSelect™ VL resin	1 L	To be determined
MabSelect™ VL resin	5 L	To be determined
MabSelect™ VL resin	10 L	To be determined
PreDictor™ MabSelect™ VL plate		To be determined
MabSelect™ VL RoboColumn™		To be determined
HiTrap™ MabSelect™ VL column	1× 5 mL	To be determined
HiTrap™ MabSelect™ VL column	5 × 5 mL	To be determined
HiScreen™ MabSelect™ VL column	1 × 4.7 mL	To be determined
MabSelect™ VL validation column		To be determined
ReadyToProcess™ MabSelect™ VL NS column	1 L (80/200)	To be determined
ReadyToProcess™ MabSelect™ VL NS column	2.5 L (126/200)	To be determined
ReadyToProcess™ MabSelect™ VL NS column	5 L (178/200)	To be determined
ReadyToProcess™ MabSelect™ VL NS column	10 L (251/200)	To be determined
ReadyToProcess™ MabSelect™ VL NS column	32 L (450/200)	To be determined

Related information

Order online on product web page.

Guidance for antibody affinity chromatography
Regulatory support file
Product instructions

References

1. De Chateau M *et al.* On the interaction between protein L and immunoglobulins of various mammalian species. *Scand. J. Immunol.*1993:37; 399-405.

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