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 subclasses 1, 3, and 4.
- 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 On request 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 subclasses 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 resin was 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).

Table 2. Protein L antibody binding affinities (1)

Species	Antibody class	Affinity*
General	Kappa light chain	Strong
	Lambda light chain	No binding
	Heavy chain	No binding
	Fab	Strong
	ScFv	Strong
	Dab	Strong
Human	IgG1	Strong
Note: Human antibodies with kappa light chain subclasses 1,3, and 4 interacts with protein L.	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 subclass that contain appropriate kappa light chain.

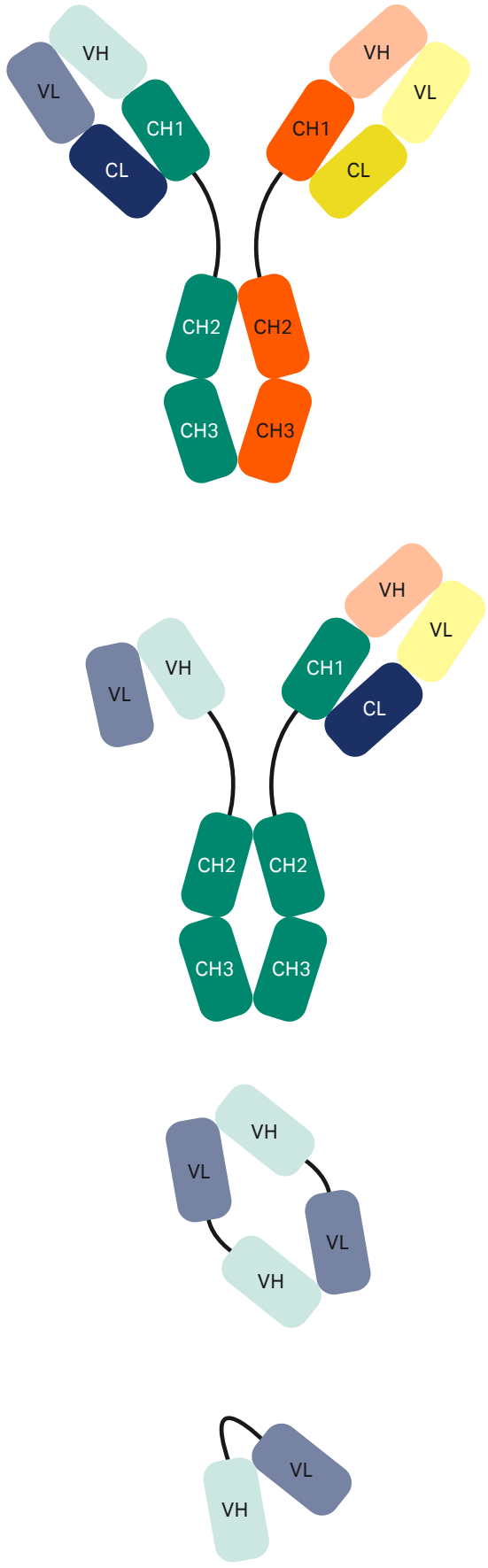

Fig 2. Antibody variant molecules. The variable light chain (VL) can have kappa or lambda variants. The protein L ligand on MabSelect™ VL resin has affinity for three of four kappa light chain variants and allows for capture of bispecific antibodies and antibody fragments.

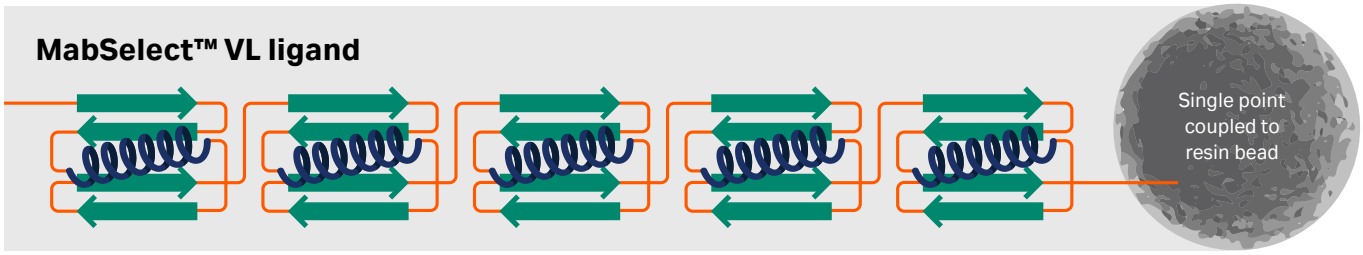

Fig 3. One domain of native protein L ligand has been genetically engineered and multimerized for better alkaline resistance on the resin bead.

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. Figure 4 shows Q_{B10} as a function of residence times for mAb (A), Fab (B) and dAb (C) on the MabSelect™ VL and Capto™ L resins. The Capto™ L resin is relatively independent of residence time for all three entities but is at a significantly lower Q_{B10} compared to MabSelect™ VL. For mAb captured with MabSelect™ VL resin, the residence time affects Q_{B10}, while the smaller entities' Q_{B10} are relatively independent of residence time. Binding capacity for mAb was 72 g/L for MabSelect™ VL resin compared to 35 g/L for Capto™ L resin at 6 min residence time. Moreover, the Q_{B10} with Fab is higher than with mAb for residence time up to 4 min. Optimum binding of mAb is reached at 10 min residence time, whereas optimum binding occurs at 6 min with Fab. For dAb, the Q_{B10} is unaffected by residence time. 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 with buffers (Fig 6), we measured relative remaining DBC of MabSelect™ VL resin over 100 CIP cycles of 15 min each with 0.1 M NaOH. Relative remaining DBC for trastuzumab was unchanged at 100 cycles. 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.

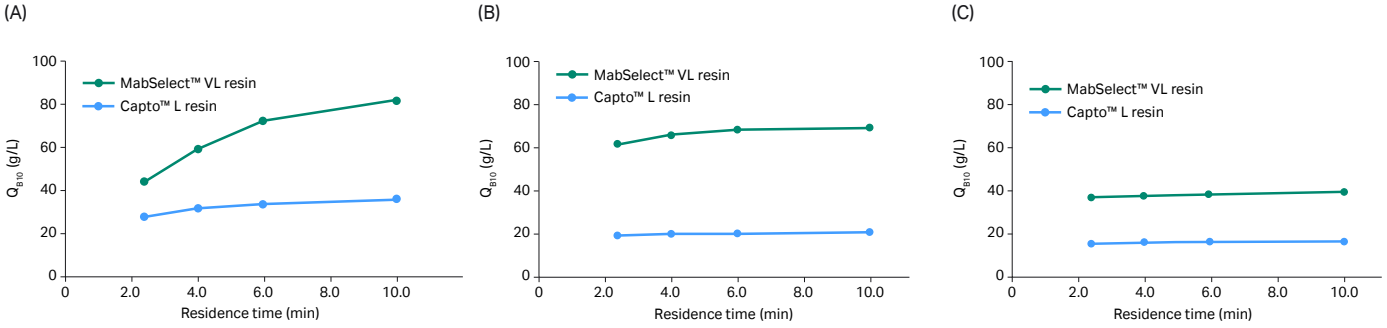

Fig 4. Q_{B10} for MabSelect™ VL and predecessor Capto™ L resins at different residence times for (A) mAb, (B) Fab, and (C) dAb.

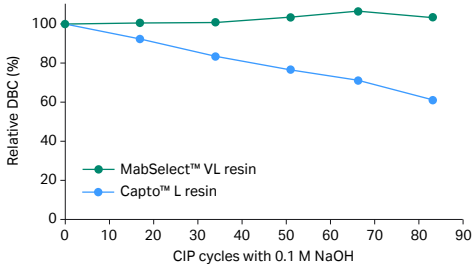

Fig 5. Accelerated alkaline-stability study of relative remaining DBC for MabSelect™ VL and Capto™ L resins over 80 cleaning-in-place (CIP) cycles with 0.1 M NaOH.

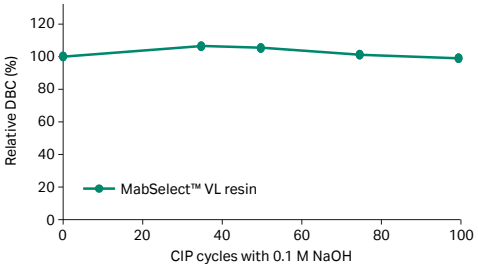

Fig 6. CIP cycling study of relative remaining DBC to test alkaline stability for MabSelect™ VL resin over 100 CIP cycles of 15 min each with 0.1 M NaOH.

Determining ligand leakage

The MabSelect™ VL ligand can be analyzed using immunoassays with commercially available antibodies for protein L. We developed an immunoassay protocol for determining the MabSelect™ VL ligand leakage in Herceptin mAb eluates using Gyrolab® technology (Gyros Protein Technologies) and commercially available antibodies for protein L. Prior to analysis, we use heat treatment with a protein complex dissociation (PCD) diluent for both the standard stock solution (MabSelect™ VL resin ligand diluted with the relevant mAb) and the eluate sample. After heat treatment, an addition of 1 % polyvinylpyrrolidone (PVP-40)-solution in a 1:1 ratio is added. Further dilutions are made with a 2:3-mix of PCD diluent and 1 % PVP-solution to generate the standard curve points and dilute the eluate sample at least to 1:4 or more. Quantification of the eluate sample is then made against the standard curve for MabSelect™ VL resin ligand. For further information on commercially available antibodies for MabSelect™ VL ligand and to request free MabSelect™ VL 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 largescale columns.

Capture of bispecific antibodies and removal of product-related impurities

Purification of antibody variants has additional challenges compared to working with conventional mAbs. Product-related impurities due to mispairing and half antibodies add complexity to the purification process. The similarities between these impurities present extra challenges in the downstream process, especially post-capture. Removing product-related impurities at the capture step using differences in avidity can help simplify the polishing steps.

MabSelect™ VL protein L resin binds to human kappa light chain subclasses 1, 3, and 4 of monoclonal antibodies. To determine the resin's ability to separate kappa-lambda heterodimer bispecific from homodimers in a feed solution a pH gradient and a step elution purification run were performed.

The feed composition comprised of a kappa light chain, trastuzumab kappa class 1 anti-HER2 light chain (1 and 2); a lambda light chain, avelumab lambda class 2 anti PDL1 light chain; and a Fc chain, anti-HER2 heavy chain (1 and 2). They were targeted to express in a ratio of 30:30:40, respectively.

Approximately 5 mg of bispecific cell culture harvest was applied to a Tricorn™ 5/100 column with MabSelect™ VL resin. The elution pH for the bispecific kappa-lambda heterodimer and the mispaired kappa homodimer was determined with a gradient elution from pH 5 to pH 2.5. This was followed by performing a stepwise pH elution, with pH obtained from the gradient elution, to separate bispecific kappa-lambda heterodimers from kappa-kappa and lambda-lambda homodimers.

The fractions were collected during the elution gradients and analyzed with SEC and LC-MS to confirm the separation of the different entities.

The step elution was demonstrated with different column formats for MabSelect™ VL resin. A 2.5 mg sample of bispecific cell culture harvest was applied to a HiTrap™ 1 mL column, and a 12 mg sample was applied to a HiScreen™ column. Step elution was performed based on the outcome from the gradient pH elution on the Tricorn™ 5/100 column format with MabSelect™ VL resin.

Figure 7 shows a chromatogram for capture of a bispecific antibody using MabSelect™ VL resin and gradient elution. The mispaired lambda homodimer did not bind to the column because it does not contain the kappa light chain. It 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.

The elution pH was determined by the apex of the peak in the gradient, and the step elution was performed with 50 mM citrate pH 3.1 and 3.4.

The results in Figure 8 show that stepwise pH elution allowed for full separation of bispecific kappa-lambda heterodimers from kappa-kappa and lambda-lambda homodimers.

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

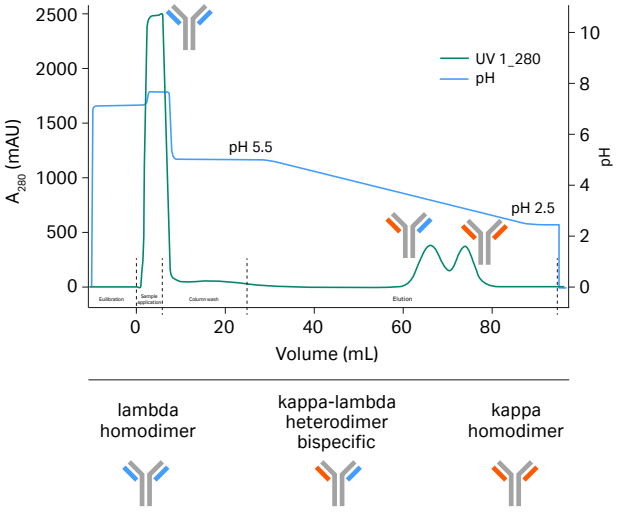

Fig 7. Chromatogram of separation of a kappa-lambda heterodimer bispecific antibody from product-related impurities.

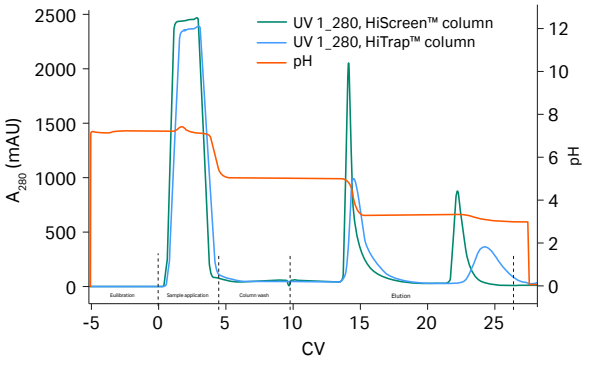

Fig 8. BsAb applied on MabSelect™ VL resin packed in a HiTrap™ 1 mL and HiScreen™ columns and eluted in a step gradient with 50 mM citrate buffer pH 3.5 and pH 3.1.

Formats for research through to commercial manufacturing

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

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 9 provides a guide for selecting an affinity chromatography resin based on target antibody variant molecule.

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.

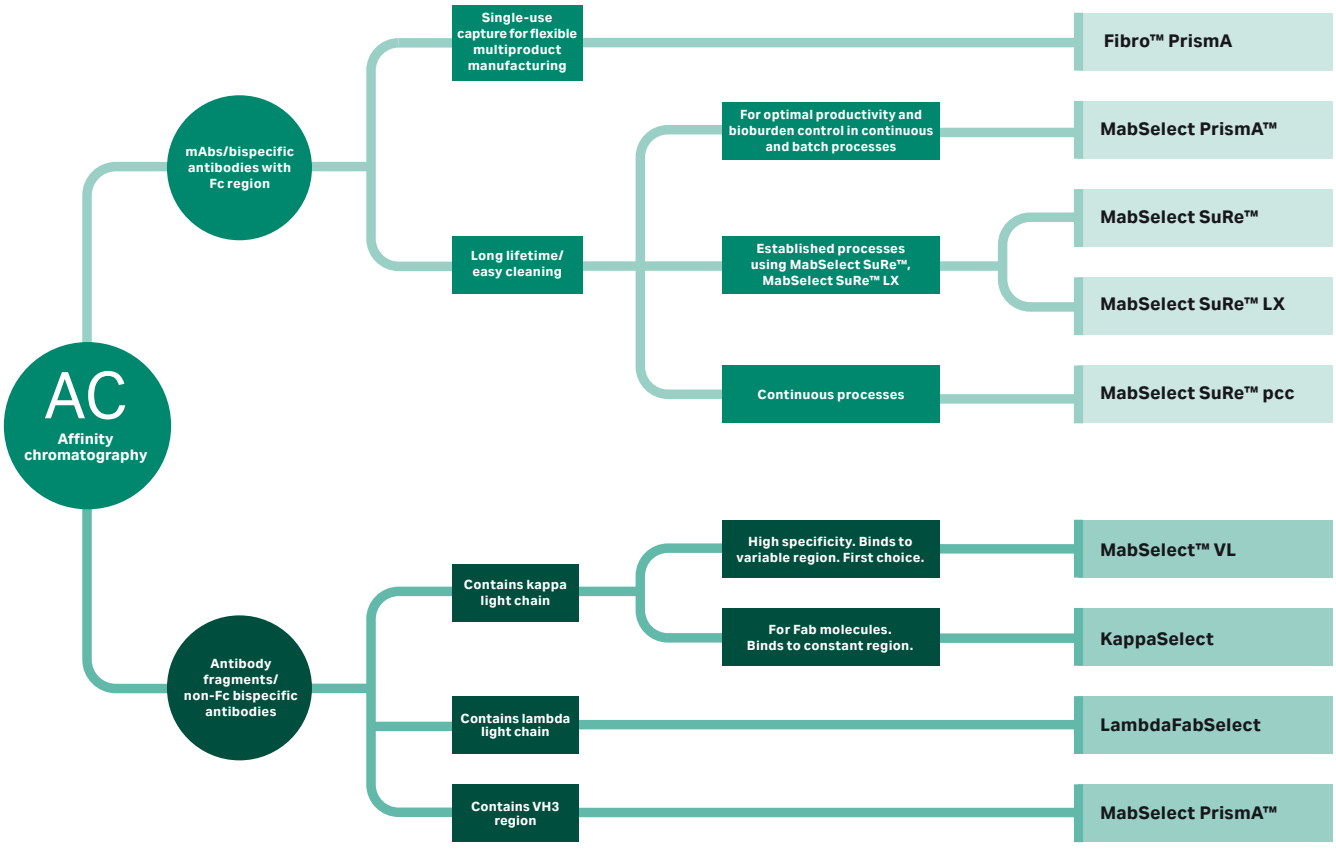

Fig 9. Selection tree for affinity chromatography (AC) resins for purification of antibody variants.

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
HiTrap™ MabSelect™ VL column	1 × 5 mL	17542053
HiTrap™ MabSelect™ VL column	5 × 5 mL	17542054
HiScreen™ MabSelect™ VL column	1 × 4.7 mL	17542015
MabSelect™ VL resin	25 mL	17542001
MabSelect™ VL resin	200 mL	17542002
MabSelect™ VL resin	1 L	17542003
MabSelect™ VL resin	5 L	17542004
MabSelect™ VL resin	10 L	17542005
PreDictor Robocolumn™ MabSelect™ VL, 200 µL	1 × 8 columns	17542033
PreDictor Robocolumn™ MabSelect™ VL, 600 µL	1 × 8 columns	17542034
PreDictor™ MabSelect™ VL plate	2 µL resin/well	17542030
PreDictor™ MabSelect™ VL plate	20 µL resin/well	17542031
PreDictor™ MabSelect™ VL plate	50 µL resin/well	17542032
MabSelect™ VL validation column	15.7 mL (10/200)	17542070
ReadyToProcess™ MabSelect™ VL NS column	1 L (80/200)	On request
ReadyToProcess™ MabSelect™ VL NS column	2.5 L (126/200)	On request
ReadyToProcess™ MabSelect™ VL NS column	5 L (178/200)	On request
ReadyToProcess™ MabSelect™ VL NS column	10 L (251/200)	On request
ReadyToProcess™ MabSelect™ VL NS column	20 L	On request
ReadyToProcess™ MabSelect™ VL NS column	32 L (450/200)	On request

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.

cytiva.com/bioprocess

CY26149-20Apr23-DF