MabSelect™ VH3 resin

AFFINITY CHROMATOGRAPHY

MabSelect™ VH3 affinity resin uses an engineered protein A ligand that interacts only with the variable heavy (VH) chain of the VH3 sequence family of the human antibody. The traditional protein A interaction with the antibody's fragment crystallizable (Fc) region is knocked out and allows efficient separation of bispecific antibody (bsAb) and antibody fragments such as fragment antigen-binding region (Fab), single-chain variable fragment (scFv), and variable heavy domain of heavy chain (VHH) that contain the VH3 sequence family. MabSelect VH3 protein A affinity resin provides high dynamic binding capacity (DBC), excellent alkaline stability, and is part of the Cytiva antibody resin toolbox. Other high-flow, modern affinity resins in the toolbox include MabSelect PrismA™ resin, MabSelect VL resins, and Capto™ polishing resins.

Key features of MabSelect VH3 resin

- High binding capacity for bispecific antibodies and antibody fragments containing VH3 sequence family.
- Provides good resolution for product-related impurities in the capture of bispecific antibodies.
- Excellent alkaline stability: stable when cleaned with 0.5 M NaOH, minimizing risk for bioburden incidents and providing long resins lifetime.

Scientific progress and protein engineering capabilities have unleashed a large variety of antibody types and have enabled the development of new treatments for many indications. Manufacturing platform approaches used for many traditional monoclonal antibodies (mAbs) are being adapted to fit respective molecules.

To provide the required purity and yield, a chromatography resin must be selected based on the domains of the target molecule, the impurity profile, and the antibody regions that the resin interacts with. The ligand of MabSelect VH3 resin has been engineered to have affinity only for the variable region of the heavy chain (VH3). Traditional protein A resins have affinity for both the Fc region and the Fab VH3 region of human antibodies. In the ligand used in the MabSelect VH3 resin, the Fc interaction has been knocked out, and VH3 interaction has been enhanced. In bioprocessing, an affinity ligand with single interaction to the Fab VH3 region is beneficial over a dual interaction affinity ligand as separation of unwanted mispaired antibodies and fragments from target bsAb may be more efficient. The VH3 sequence family is the most common VH class for antibodies in commercialized biologics.

Fig 1. MabSelect VH3 resin is available in bulk and in prepacked columns.

Table 1. Main characteristics of MabSelect VH3 resin

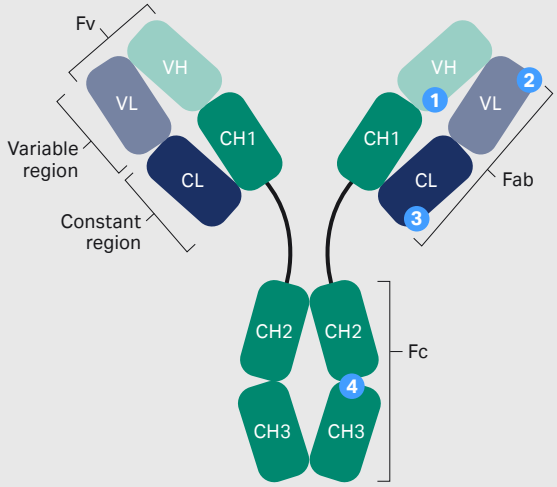
Matrix	Highly cross-linked agarose, spherical
Ligand	Alkaline stabilized protein A-derived (*E. coli*), no interaction with the Fc region and enhanced interaction with the VH3 region
Ligand coupling	Single-point attachment
Coupling chemistry	Epoxy
Particle size d50v*	~ 60 µm
DBC Q_{B10} †	~ 70 mg mAb/mL resin at 6 min residence time ~ 60 mg mAb /mL resin at 4 min residence time
Recommended maximum operating flow velocity	300 cm/h‡
pH stability, operational§	3 to 12
pH stability, CIP¶	2.5 to 13.7
Delivery conditions	20% ethanol

* Median particle size of the cumulative volume distribution
† 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 HiScreen™ column at 10 cm bed height for mAb in PBS buffer, pH 7.4
‡ 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
§ pH range where resin can be operated without significant change in function
¶ pH range where resin can be subjected to cleaning-in-place (CIP) without significant change in function

Capture

Affinity chromatography

Select affinity resins for your monoclonal antibody, multispecific antibody, or antibody fragments based on the domains in the target molecule and the impurity profile.



Protein A ligand

1† 4 MabSelect PrismA resin
Fibro™ PrismA fiber units

1 MabSelect VH3 resin

4 MabSelect SuRe™ resin
MabSelect SuRe LX resin

Protein L ligand

2* MabSelect VL resin

Other protein ligands

3 KappaSelect resin
LambdaFabSelect resin

* *Variable region of a human antibody's kappa light chain subtypes 1, 3, and 4 interacts with protein L.*

† *VH3 sequence interacts with protein A.*

Polishing 1

Cation exchange chromatography (CIEX) or multimodal CIEX

Select Capto resins

Polishing 2

Anion exchange chromatography (AIEX) or multimodal AIEX

Select Capto resins

Fig 2. Select affinity resin based on the interactions with the target molecule and impurities. Remove as much impurities as possible in the capture step. Then continue purification with one or two polishings steps.

Select resins based your target antibody and impurity profile

When selecting affinity resins, you should base your choice on the domains in your target molecule and the impurity profile. This applies whether working with mAbs, bsAb, multispecific antibody, or antibody fragments. The affinity ligands coupled on the chromatography resin beads interact specifically to different domains of the antibodies. For conventional mAbs, protein A is used for dual interactions (Fc and VH3 sequence of the VH region). But when separating antibody variants and impurities, you may need resins designed for other types of interactions. Figure 2 shows how different Cytiva chromatography resins interact with various antibody domains. Together, they provide a toolbox of resins for purifying antibody variants.

Specific binding for VH3 sequence family

MabSelect VH3 resin provides good separation of mispaired antibodies, half antibodies, and fragments as it is highly specific for VH3 class of antibodies and does not bind VH1 or VH2 class antibodies, or the antibody Fc region or light chains. Figure 3 demonstrates the selectivity of MabSelect VH3 resin. We loaded three VH1 and one VH2 class of antibody (5 mg each) onto a Tricorn™ 5/50 column packed with MabSelect VH3 resin.

Figure 3 shows that none of the VH1 and VH2 antibodies are bound to the MabSelect VH3 resin and instead passed through the column during sample application phase. Furthermore, no elution of antibody occurs in the elution phase. This experiment also demonstrates that MabSelect VH3 resin has no affinity for Fc region and light chains of an antibody. However, we always recommend screening for resins.

The MabSelect VH3 resin does not bind to all VH3 class of antibody molecules or fragments due to the huge sequence variation in VH3 gene. MabSelect VH3 resin binds similar VH3 sequence variations as MabSelect PrismA resin.

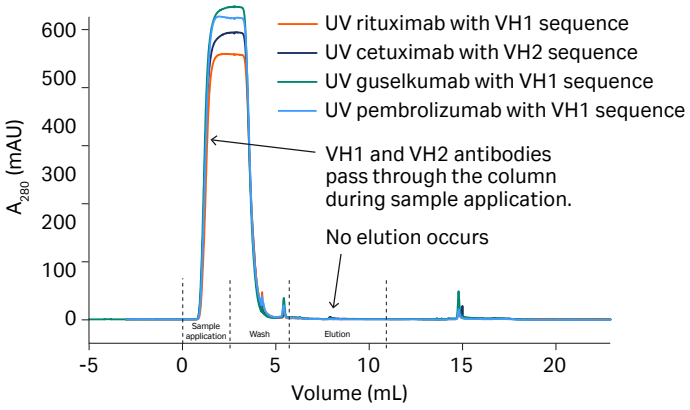


Fig 3. Chromatograms showing no binding of VH1 and VH2 class of mAbs to MabSelect VH3 resin. All mAbs passed though the column in the flowthrough.

High binding capacity for different antibody variants

High DBC allows high mass throughput of processed antibody per resin volume unit. It also provides productivity and process-economy benefits. The DBC of MabSelect VH3 resin for VH3-containing regular mAb, Fab, and VHH was evaluated at different residence times (RT) at 10% breakthrough (Q_{B10}) by frontal analyses using ÄKTA pure™ 25 system complemented with UNICORN™ evaluation software. MabSelect PrismA resin was included as well. HiScreen columns at 10 cm bed height were packed with both types of resins.

For regular mAb (of VH3 class of VH domain), MabSelect VH3 resin shows similar binding capacity as MabSelect PrismA resin (Fig 4A). For example, at 6 min RT, MabSelect VH3 resin and MabSelect PrismA resin showed a DBC of 70 g/L and 72 g/L, respectively. MabSelect VH3 resin showed significantly higher (70%) binding capacity for Fab compared to MabSelect PrismA resin. Interestingly, the optimal binding capacity seems to be reached at 2.4 min RT with a DBC of 54 g/L (Fig 4B). For VHH, MabSelect VH3 resin showed a higher DBC (11 to 14%) compared to MabSelect PrismA resin. The MabSelect VH3 resin showed a DBC of 36 to 39 g/L for VHH at tested RTs (Fig 4C). Binding capacity for VHH is relatively unaffected by RT similar to the results for Fab. The higher binding capacity, particularly at short RT, provides productivity advantages in biomanufacturing.

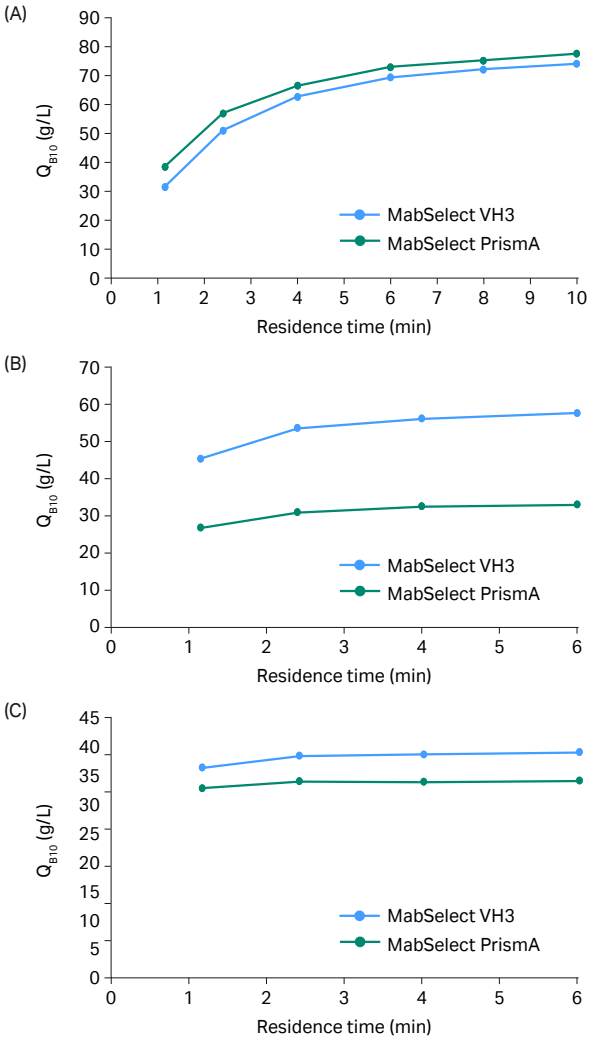


Fig 4. DBC at Q_{B10} for MabSelect VH3 and MabSelect PrismA resins at different RTs for mAb (A), Fab (B), and VHH (C).

Purification performance of MabSelect VH3 resin

We investigated purification performance of MabSelect VH3 resin by studying yield, pool volume, mAb aggregates, clearance of host cell protein (HCP), and leached ligand. In this investigation, 20 mL of clarified cell culture harvest containing 2.46 g/L trastuzumab containing the VH3 sequence family was loaded at 6 min RT to a Tricorn 5/50 (1 mL) column. Bound mAb was eluted in 50 mM sodium acetate, pH 3.5 (Fig 5), with a yield of 98% mAb. The pool volume was very low and in line with other MabSelect resins. The pool contained 0.75% aggregates, 4 ppm of leached ligand, and 251 ppm HCP, a reduction factor of HCP of about 600 times (from about 1.5×10^5 to 250 ppm) (Table 2). Ligand leakage and HCP were measured using Gyrolab technology (Gyros Protein Technologies Group). A commercial antibody for protein A was used for ligand leakage, and CHO-HCP kits were used for HCP analysis.

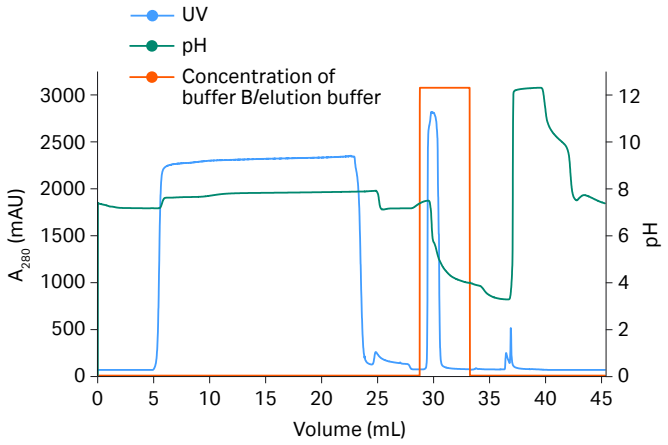


Fig 5. Purification of mAb with MabSelect VH3 resin.

Table 2. HCP, leached ligand, and mAb aggregates in elution pool in purification of mAb from cell culture harvest on MabSelect VH3 resin

Load	80% of DBC at Q_{B10}; 49.2 mg
Yield (%)	98
Pool volume (column volumes [CV])	1.45
Pool concentration (mg/mL)	33.2
Aggregates (%)	0.72
HCP* (ppm)	251
Protein A (ppm)	4

* HCP at start 153 335 ppm.

Robust resin designed for harsh cleaning over many cycles

High DBC is not only important at the beginning of the resin's lifetime. The resin needs to withstand harsh cleaning conditions to reduce risk for bioburden incidents and carry over between batches. The DBC must be maintained over many cycles to allow for high productivity.

The alkaline stability of the MabSelect VH3 resin was evaluated in an accelerated alkaline stability study (Fig 6) and in a repeated CIP cycling study (Fig 7) with 0.5 M NaOH. In the accelerated alkaline stability study, MabSelect VH3 resin was packed into Tricorn 5/50 column and exposed to 0.5 M NaOH for 4 hours, corresponding to 16 CIP cycles of 15 min contact time. A total of six rounds of incubation were performed. DBC at every 16th cycle was measured with purified mAb (trastuzumab) or VHH. The relative remaining binding capacity (in %) was calculated between incubation periods where the initial DBC (at 0 cycles, no treatment with NaOH) was considered to be 100%. After 96 cycles, the relative remaining binding capacity of MabSelect VH3 resin for both mAb and VHH was found to be 93%.

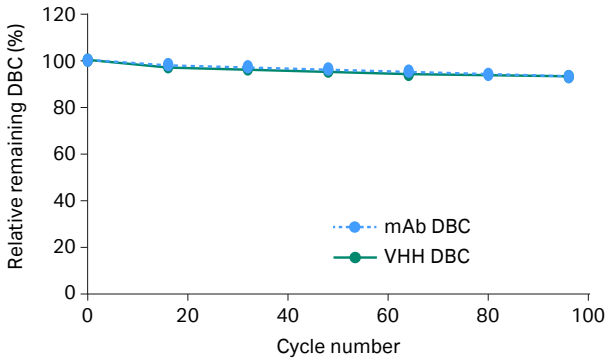


Fig 6. Relative remaining binding capacity (Q_{B10}) of MabSelect VH3 resin for mAb and VHH after 96 CIP cycles with 0.5 M NaOH in an accelerated alkaline stability study.

The repeated CIP cycling study was also performed on a Tricorn 5/50 column. Each cycle includes all steps in a purification process but uses buffer instead of an antibody feed. The column was subjected to repeated nine cycles with 2 CV of PBS, pH 7.4; 5 CV of 50 mM sodium acetate, pH 3.5; and 3 CV of 0.5 M NaOH (contact time 15 min/cycle), and the DBC was measured at every 10th cycle with mAb (trastuzumab). The study was performed for 200 cycles. The relative remaining DBC was calculated as mentioned above. At 100 cycles, the remaining DBC for mAb is similar, as seen in accelerated alkaline stability study (93%). After 200 cycles, the remaining DBC is found to be about 84% (Fig 7), showing an excellent alkaline stability.

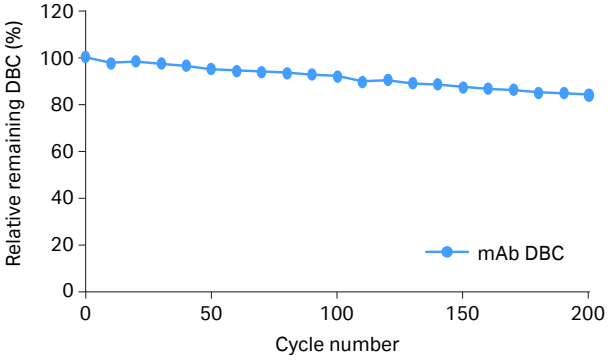


Fig 7. Relative remaining binding capacity (Q_{B10}) of MabSelect VH3 resin for mAb after 200 repeated cycles including 15 min CIP cycle with 0.5 M NaOH.

Lifetime study with mAb-containing sample and 0.5 M NaOH for CIP

In this study, a Tricorn 5/50 (1 mL) column was packed with MabSelect VH3 resin. The column was subjected to repeated cycles of loading of cell culture harvest containing VH3 class mAb (trastuzumab) at 6 min RT up to 80% of DBC at Q_{B10}. Bound mAb was eluted in 50 mM sodium acetate, pH 3.5, followed by a strip of column with 100 mM acetic acid, pH 2.9. CIP was performed with 0.5 M NaOH (15 min contact time) after each elution phase. The study was performed for 100 cycles. DBC at Q_{B10} was determined at every 10th cycle by frontal analysis using ÄKTA pure 25 chromatography system complemented with UNICORN software.

As seen in Figure 8, the remaining DBC after 100 purification cycles was 92%. As expected, the lifetime study demonstrated very stable yield, elution pool volume, and pool concentration. The pool volume was low and in line with other MabSelect resins. Aggregate concentrations in elution pools were low. Concentrations of HCP and leached protein A were stable and low with no significant trend. Table 3 summarizes lifetime performance data from every 10th cycle.

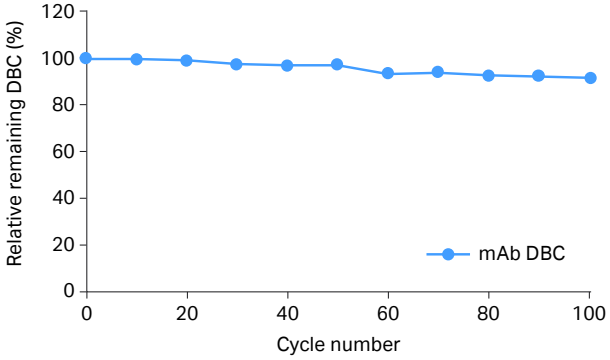


Fig 8. Relative remaining binding capacity (Q_{B10}) of MabSelect VH3 resin for mAb after 100 repeated purification cycles including 15 min CIP cycle with 0.5 M NaOH.

Table 3. Purification performance data of MabSelect VH3 resin obtained from a lifetime study of 100 cycles mAb purification from cell culture harvest containing VH3 class mAb. Each purification cycle includes 15 min CIP with 0.5 M NaOH.

Cycle number	Yield (%)	Pool volume (CV)	Pool concentration (mg/mL)	Aggregates (%)	HCP (ppm)	Protein A (ppm)
1	95	2.25	20.67	0.43	276	9
10	94	1.80	25.64	0.42	301	4
20	97	1.83	26.16	0.73	241	4
30	90	1.84	24.15	0.83	250	8
40	95	1.90	24.71	0.45	223	6
50	94	1.84	25.21	0.89	216	8
60	93	1.85	24.62	0.63	246	8
70	93	1.87	24.34	0.61	199	9
80	92	1.87	24.26	1.08	230	10
90	91	1.89	23.78	0.53	251	9
100	93	1.94	23.49	0.30	158	7

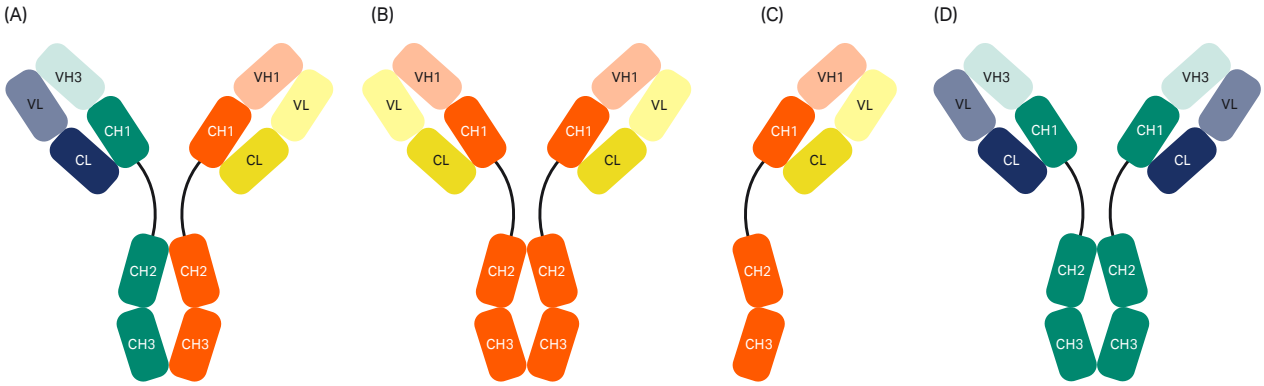


Fig 9. An asymmetric bispecific antibody with VH3 sequence on one heavy chain and VH1 sequence on the other heavy chain (A) can be purified using MabSelect VH3 resin. Product-related impurities that contain only VH1 sequence family (B) and (C) will end up in the flowthrough. Homodimers with VH3 on both heavy chains (D) will elute later than the target bsAb (A) due to avidity effects.

High resolution for bispecific antibody provides efficient purification

Asymmetric bispecific antibodies can form product-related impurities such as homodimers, half antibodies, and excess of light chains during cell culture. They are also prone to aggregation. The similarities between these impurities present extra challenges for downstream processes, especially post-capture. MabSelect VH3 resin and its novel selectivity enables separation in the capture step of asymmetric bispecific antibodies where only one of the heavy chains includes the VH3 sequence family from product-related entities (Fig 9).

To demonstrate the potential of MabSelect VH3 resin to separate bispecific antibody from product-related impurities, a cell culture harvest containing transient expressed heterodimeric bispecific antibody (emicizumab, VH1:VH3), and two mispaired homodimeric species (VH1:VH1 and VH3:VH3) was loaded onto the MabSelect VH3 column. Because MabSelect VH3 resin does not have the Fc interaction, the homodimer VH1:VH1 will not bind and therefore goes to the flowthrough. The VH3:VH3 homodimer binds with two interactions with higher avidity compared to the correctly paired VH1:VH3 heterodimer and therefore elutes at a lower pH. By eluting in a gradient or in a two-step elution with different pH values, a separation between VH1:VH3 and VH3:VH3 could be feasible. In a production scenario, you will elute the bispecific during a step elution and separate unwanted homodimer during the strip of the column.

Approximately 6 mg of bispecific in cell culture harvest was applied onto a Tricorn 5/100 column packed with MabSelect VH3 resin. A 20 CV gradient elution from 20 mM sodium citrate pH 6.0 to 20 mM sodium citrate pH 2.5 was performed. As seen in Figure 10, homodimer VH1:VH1 did not bind to the column and was recovered in the flowthrough. Heterodimeric bispecific VH1:VH3 and mispaired VH3:VH3 bound to the column but eluted at two distinct peaks with good resolution, as expected. The elution pH of the heterodimer and homodimer is found to be pH 4.0 and pH 3.8, respectively. The delta pH of hetero- and homodimer for the molecule tested is only about 0.15 pH units, and a baseline separation with gradient elution is not likely. Step elution with optimized condition could facilitate baseline separation (the high load run below is an example). In production, step elution is preferred over gradient elution. Flowthrough and fractions corresponding to each peak were collected and subjected to liquid chromatography-mass spectrometry (LC-MS) analyses that further confirmed the separation of different species.

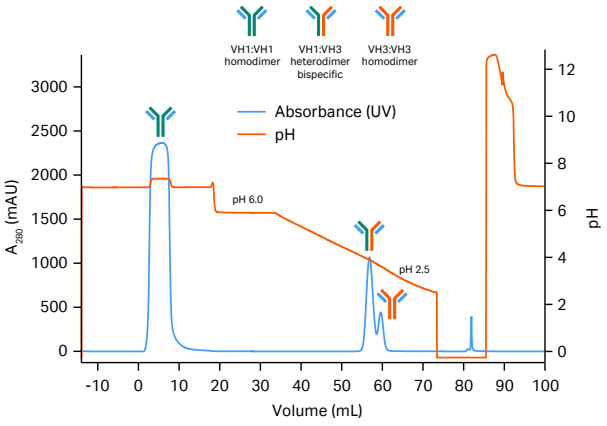


Fig 10. Separation of heterodimer bispecific antibody from product-related impurities on a Tricorn 5/100 column packed with MabSelect VH3 resin.

Efficient separation of bispecific antibody with high load and step elution was also demonstrated. In this experiment, the load of bispecific in cell culture harvest on MabSelect VH3 resin was 30 mg bsAb/mL resin. Two-step elution was performed at pH values found in the experiment above, pH 4.0 for eluting target heterodimer and pH 3.5 for eluting homodimer. The same column format as in the previous experiment was used. The bispecific heterodimer was found well separated even at this high load run (Fig 11), demonstrating the high binding capacity of the resin for bispecific antibody without compromising resolution. Again, collected fractions were subjected to LC-MS analyses.

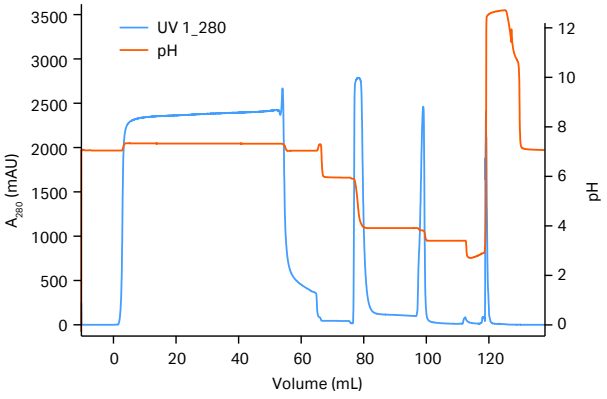


Fig 11. A 30 mg bsAb-containing feed applied per mL MabSelect VH3 resin packed into Tricorn 5/100 column demonstrates the separation of heterodimer bispecific antibody from product-related impurities with step elution at a high load run.

Determining ligand leakage

Purification of antibody-based molecules using a chromatography resin and protein affinity ligand introduces leaked ligand into the process. Thus, measuring ligand leakage with a properly calibrated assay is important for controlling the production process and drug product quality. We recommend the VH3 ligand ELISA kit (product code 29737000) for this purpose, as it was designed for use specifically with MabSelect VH3 protein A resin. The kit provides parts per billion (ppb) sensitivity and high drug product tolerance up to 30 g/L. It is supplied with all the reagents needed to perform the assay, including a resin-matched VH3 ligand standard to calibrate your assay for the most accurate results.

The ligand is also available under non-transferable limited license for analytical purposes for protein A leakage qualification. Contact us for further information.

Base matrix properties

The agarose high-flow 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 in different bed heights. Learn more in the application article Chromatography column packing - MabSelect PrismA resin.

Recommended protocol for MabSelect VH3 resin

You can use the same buffers for MabSelect VH3 resin as for MabSelect PrismA protein A resin, with minor modifications. Equilibrate the column with 20 mM sodium phosphate, 0.15 M NaCl, pH 7.2. After sample application, wash the column with a low-salt buffer (e.g., equilibration buffer) and then do a high-salt wash with 20 mM sodium phosphate, 0.5 M NaCl, pH 7.0 to remove impurities such as HCP. To remove high salt, it is important to wash the column with buffers of pH ≥ 6.5. With lower pH, there is a risk for washing off the target antibody. Elute the sample in 50 mM sodium acetate, pH 3.5. Preform an acid strip in 100 mM acetic acid (2 CV) and CIP with 0.5 M NaOH (3 CV with 15 min contact time).

High-salt intermediate washes of protein A resins can cause antibody leakage when the target molecule has a single VH3 interaction with the ligand, as do some bsAbs and antibody fragments (such as Fabs or VHH). If you observe leakage, we recommend lowering the salt concentration in the intermediate wash. However, if a high-salt wash is needed, we recommend determining the Q_{B10} in the presence of the intended salt concentration and then loading 80% of the Q_{B10} on the column.

Resin storage

Store unused MabSelect VH3 resin in its container between 2 to 8°C. Ensure that the screw top is fully tightened. Equilibrate packed columns in buffer containing 20% ethanol or 2% benzyl alcohol. After storage, equilibrate with starting buffer and perform a blank run, including CIP, before use.

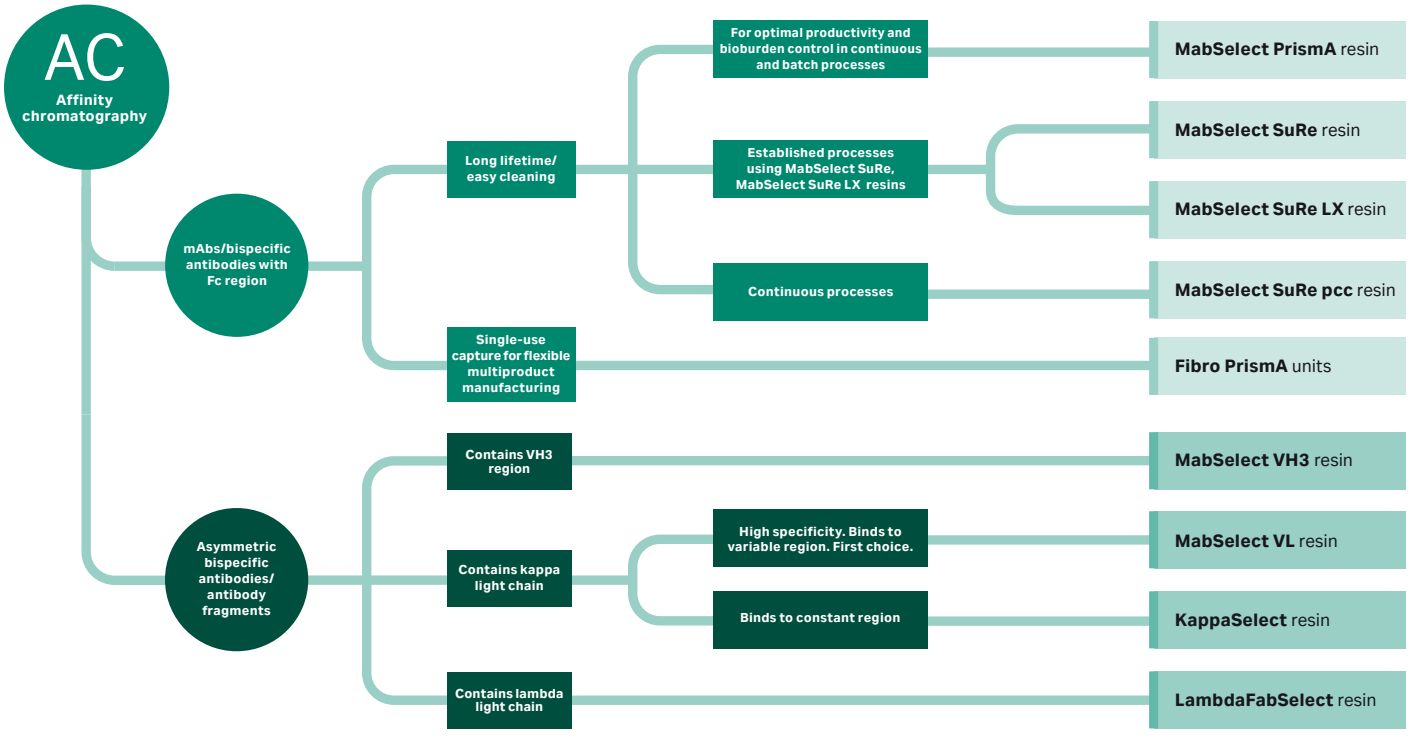


Fig 12. Selection tree for affinity chromatography resins for purification of antibodies.

A capture chromatography toolbox for antibody variants

Affinity chromatography separates proteins based on 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 12 provides a guide for selecting an affinity chromatography resin based on your 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. We continue to make significant investments in capacity expansion and supply stability for a reliable and consistent supply of our chromatography resins and prepacked ReadyToProcess™ columns. We recommend customers work closely with your Cytiva representative 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, we have created a strategic reserve of chromatography resins used in approved manufacturing processes. Resin types, volumes, and storage locations of the reserve are regularly reviewed to ensure effective deployment of materials globally.

Support and training

MabSelect VH3 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.

Use Cytiva online learning to build your skills and continue your education.

Ordering information

Product	Size	Product code
HiTrap™ MabSelect VH3	1 × 1 mL	17549351
HiTrap MabSelect VH3	5 × 1 mL	17549352
HiTrap MabSelect VH3	1 × 5 mL	17549353
HiTrap MabSelect VH3	5 × 5 mL	17549354
HiScreen MabSelect VH3	1 × 4.7 mL	17549315
MabSelect VH3 resin	25 mL	17549301
MabSelect VH3 resin	200 mL	17549302
MabSelect VH3 resin	1 L	17549303
MabSelect VH3 resin	5 L	17549304
MabSelect VH3 resin	10 L	17549305
PreDictor™ RoboColumn MabSelect VH3, 200 µL	1 × 8 columns	17549333
PreDictor RoboColumn MabSelect VH3, 600 µL	1 × 8 columns	17549334
PreDictor MabSelect VH3, 2 µL	1 × 4 plates	17549330
PreDictor MabSelect VH3, 20 µL	1 × 4 plates	17549331
PreDictor MabSelect VH3, 50 µL	1 × 4 plates	17549332
MabSelect VH3 validation column	1 × 15.7 mL (10/200)	17549370
ReadyToProcess MabSelect VH3 columns	Contact us for sizes and product codes	
VH3 ligand ELISA kit	1 kit	29737000

Please contact us to request samples of MabSelect VH3 resins.

Related information

Product instructions
Regulatory support file
Guidance for antibody affinity chromatography
Guide: Select affinity chromatography resin for your antibody
eLearning: mAb variants
eLearning: purification of mAb variants
VH3 ligand ELISA kit

cytiva.com

For local office contact information, visit cytiva.com/contact

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