MabSelect Xtra

AFFINITY CHROMATOGRAPHY

MabSelect Xtra™ is a recombinant protein A-based affinity medium engineered to give an exceptionally high dynamic binding capacity for monoclonal antibodies. In addition, MabSelect Xtra is optimized for Fc-fusion proteins. Higher capacity translates directly to lower cost of production.

Key performance characteristics of MabSelect Xtra include:

- High dynamic binding capacity and suitable for high-expression feedstocks
- Improved process economics through reduced raw materials costs and/or reduced number of cycles
- Very low, unspecific binding due to high ligand selectivity and matrix hydrophilicity
- High capacity for many Fc-fusion proteins



Fig 1. MabSelect Xtra captures antibodies from high-expression feedstocks with improved efficiency and economy.

Medium characteristics

Description

MabSelect Xtra is a member of the MabSelect™ product line of recombinant protein A-based affinity media for capturing monoclonal antibodies. Like its companion product, MabSelect, MabSelect Xtra is based on an innovative, high-flow agarose base matrix, optimized for maximum capacity (Fig 2). The base matrix for MabSelect Xtra has increased porosity and decreased particle size relative to MabSelect. The recombinant protein A is coupled to the base matrix at the C-terminal cysteine via a stable thio-ether bond. It is the same ligand and coupling chemistry used in MabSelect. This oriented coupling contributes to the increased capacity seen with these media.

MabSelect family

The MabSelect family of media for process-scale purification of monoclonal antibodies comprises MabSelect, MabSelect Xtra, MabSelect SuRe™, and MabSelect SuRe LX. MabSelect is designed for high-throughput purification of monoclonal antibodies from large volumes of feed. MabSelect SuRe is composed of the same rigid, high flow agarose matrix, but posesses an alkali-stabilized rProtein A-based ligand. The MabSelect SuRe ligand provides greater stability than conventional rProtein A-based media and allows extended use of 0.1–0.5 M NaOH for cleaning-in-place. MabSelect SuRe LX has been further developed from MabSelect SuRe to give even higher binding capacity at longer residence time. For more information on MabSelect, MabSelect SuRe, and MabSelect SuRe LX, refer to Data files 18-1149-94, 11-0011-65, and 28-9870-62, respectively.

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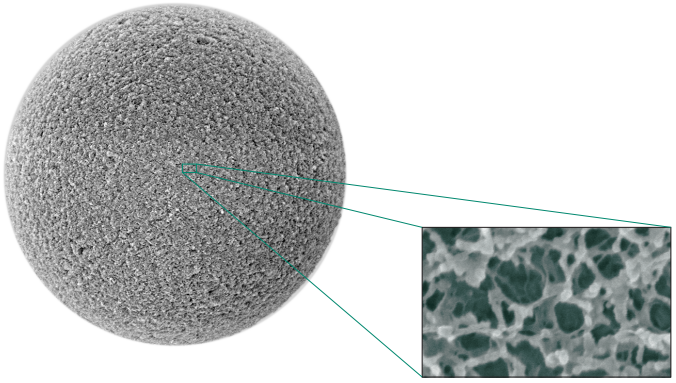


Fig 2. Electron micrographs of a MabSelect Xtra particle. The inset (resolution 500 nm) shows the structure of the high-flow agarose matrix with large pores and high connectivity.

MabSelect Xtra is easy to pack and operate in a wide variety of columns from laboratory to production scale. Well-proven packing and cleaning-in-place (CIP) recommendations are available, as are scale-up studies.

Table 1 lists the key characteristics of MabSelect Xtra.

Table 1. Main characteristics of MabSelect Xtra

Composition	Highly cross-linked agarose
Average particle size[1]	d_{50v} ≈ 75 µm
Ligand	Recombinant protein A (*E. coli*)
Coupling chemistry	Epoxy
Dynamic binding capacity[2]	Approx. 40 mg human IgG/mL medium (MabSelect approx. 30 mg IgG/mL medium)
Chemical stability	Stable in all aqueous buffers commonly used in protein A chromatography:
	0.1 M 20% sodium citrate/HCl (pH 3), 6 M Gua-HCl, 6 M urea, 20% ethanol, 2% benzyl alcohol
Recommended pH	
Working range	3–10
Cleaning-in-Place (CIP)	2–12 (see Cleaning and sanitization)
Recommended mobile phase velocity[3]	100–300 cm/h (MabSelect 100–500 cm/h)
Temperature stability[4]	2°C to 40°C
Delivery conditions	20% ethanol
Mammalian product	No material of animal origin is contact-free used in the manufacturing process
Regulatory support	Regulatory Support File available

[1] d_{50v} is the median particle size of the cumulative volume distribution.
[2] Determined at 10% breakthrough by frontal analysis at a nominal mobile phase velocity of 250 cm/h in a column with a bed height of 10 cm (2.4 min residence time).
[3] In a packed bed, the maximum recommended operating velocity for MabSelect Xtra is 300 cm/h at 20 cm bed height, with water at 20°C. Operating pressure is less than 2 bar.
[4] Recommended long-term storage conditions: 2°C to 8°C, 20% ethanol.

Dynamic binding capacity

Lower cost of production is a key processing goal of monoclonal antibody manufacturers. In upstream operations, cell culture expression levels are steadily increasing and have reached multi-gram levels per liter medium in many processes.

MabSelect Xtra responds to these improvements in cell culture optimization; the medium is designed for the downstream capture of antibodies from high expression feedstocks. Confirmation of the high dynamic binding capacity (DBC) of MabSelect Xtra compared with competitor high capacity media is seen in Figure 3, where the two media were tested with human IgG.

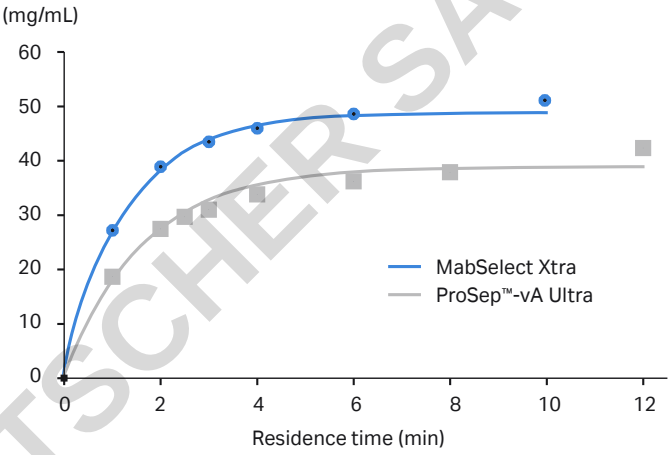


Fig 3. At equivalent residence times, MabSelect Xtra has 30% higher dynamic binding capacity than porous, glass-based protein A medium. Feed concentration was 1.1 mg/mL human polyclonal IgG. Data obtained in studies performed at Cytiva laboratories

The outstanding dynamic binding capacity of MabSelect Xtra is also observed when two different monoclonal antibody feedstocks are tested. At a residence time of 2.4 min the dynamic binding capacity at 10% break-through of a humanized IgG_4 (MAb 1) is 42 mg/mL and of a humanized IgG_1 (MAb 2) about 35 mg/mL. Increasing the residence time to 4.8 min, increases the dynamic binding capacity by 15% (Fig 4).

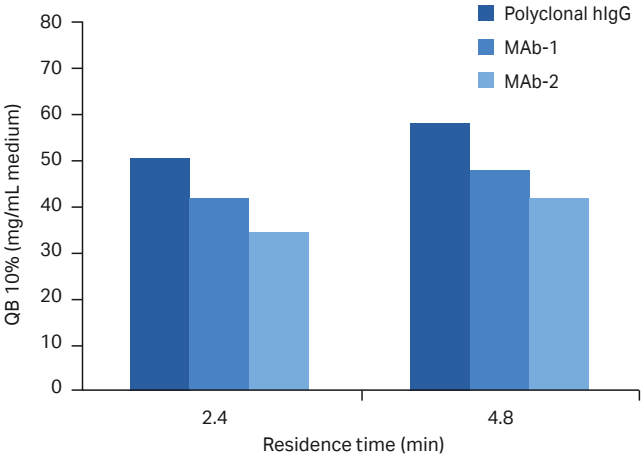


Fig 4. MabSelect Xtra dynamic binding capacity of immunoglobulins at 2.4 min residence and 4.8 min, respectively.

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Protein A leakage

Leakage of protein A from MabSelect Xtra is generally low. However, leakage of protein A is also affected by chromatographic running conditions and the composition of feed stock.

A purification study of IgG_4 at pilot scale, which included a blank run and CIP showed leakage levels of 5.5–7.5 ppm (ng/mg), see Figure 5.

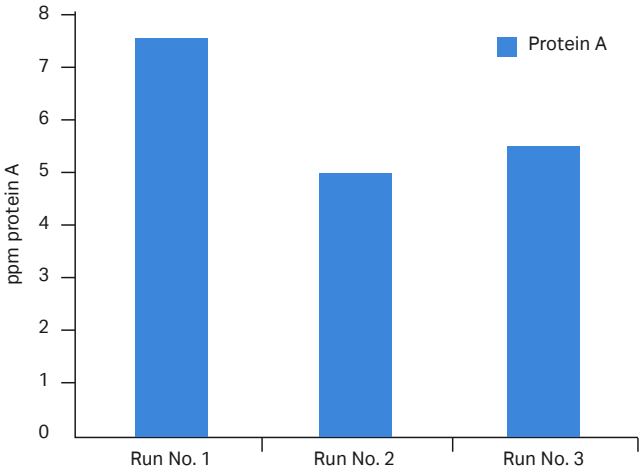


Fig 5. The leakage of protein A in the eluted IgG_4 material showed protein A levels between 5.5 and 7.5 ppm (ng protein A/mg IgG).

To remove loosely bound protein A, a blank run including CIP is recommended before the first run with antibody feed and after storage of the media.

Host cell protein study

The level of host cell proteins in the eluate from MabSelect Xtra has been assessed following repeated processing of a Chinese hamster ovary (CHO) supernatant spiked with human IgG. Complete cycles (equilibration, loading, elution, washing, and CIP) were run, after which the level of host cell protein in the eluate was measured using a high sensitivity ELISA protocol. A comparative study was made on a protein A medium attached to a porous glass base matrix.

Results show that the content of host cell protein in the eluates was relatively constant over 55 cycles (Fig 6). However, normalized levels (in ppm) were approximately 6-fold higher for a glass bead-based medium than for MabSelect Xtra. This result suggests a higher degree of nonspecific adsorption to porous glass beads compared to the hydrophilic agarose base matrix in MabSelect Xtra.

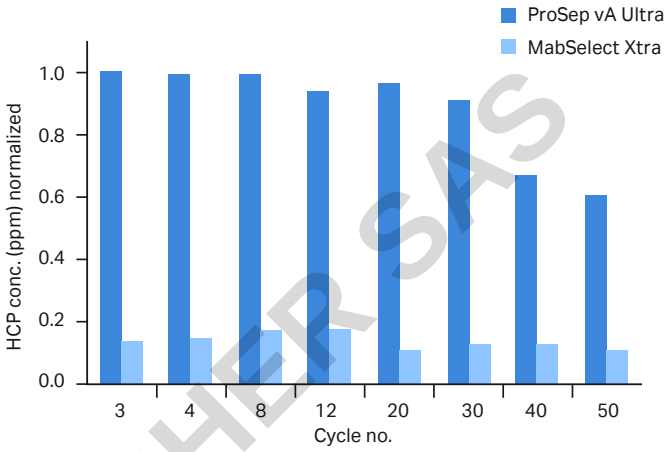


Fig 6. Normalized HCP concentration: a comparison between MabSelect Xtra and a porous glass bead, high-capacity medium. Data obtained in studies performed at Cytiva laboratories.

Operation

Method optimization

Method optimization should establish conditions that promote binding the highest amount of target antibody in the shortest time with maximum purity and recovery. In general, all antibodies of human origin, except subclass 3, have a high affinity for protein A.

To optimize elution, determine the highest pH that allows efficient desorption of antibody. Step-wise elution is preferred at large-scale as the antibody elutes in a smaller pool volume, thus decreasing buffer consumption and shortening cycle times.

PreDictor™ 96-well filter plates or miniaturized columns, prefilled with MabSelect Xtra media, are suitable for efficient high-throughput screening of chromatographic conditions during process development. Defined conditions can then be verified and optimized with HiScreen™ or HiTrap™ prepacked columns.

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Process development

For process development work using MabSelect Xtra in small-scale columns, we recommend HiScreen prepacked columns with a 10 cm bed height. Choose a residence time that meets the demands on capacity and nominal fluid velocity. Make sure the chosen bed height and velocity do not conflict with the large-scale pressure/flow limitations (see Fig 7).

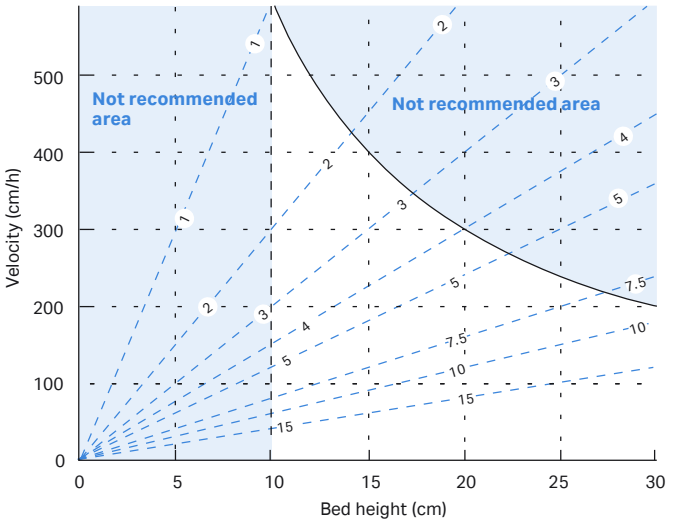


Fig 7. Operating window for MabSelect Xtra: Choosing bed height and operating velocity in terms of residence time, pressure restrictions, and large-scale column packing.

Figure 7 shows the possible operating window with combinations of bed height and operational nominal mobile phase velocity for MabSelect Xtra. The Figure also displays the residence time in minutes for any bed height and velocity. Pressure drop and packing limitations at large scale are also included. The solid line shows the large-scale column pressure restriction (light blue background). The dashed line indicates that operating below 10 cm bed height is not favorable (light blue background). The reason for this is that large diameter columns have a very different aspect ratio, and that packing short wide beds is a greater challenge.

Use Figure 7 as a guide when determining suitable bed height and operating velocity in terms of residence time and thus capacity and pressure drop.

Cleaning and sanitization

MabSelect Xtra is based on the same rProtein A ligand and same coupling chemistry as MabSelect, and similar cleaning-in-place (CIP) protocols can be applied. For MabSelect, a high-throughput screening approach using PreDictor plates has been performed [1]. The most favorable results were obtained using a two-step sequence with reducing agent (100 mM 1-Thioglycerol) followed by 15 mM NaOH. Stronger NaOH can be used, but might reduce the lifetime of the media. The two-step CIP protocol was verified in column experiments using CHO cell supernatant with humanized IgG_1. CIP was performed in every cycle using a contact time of 15 min and a blank cycle was run every 10th cycle for measuring of carryover. 110 CIP cycles were performed with consistent results and no carry-over was observed.

Of the one step CIP protocols evaluated in the screening for MabSelect, 50mM NaOH gave best results but this solution might reduce the lifetime of the media. Addition of salt (e.g., NaCl och Na_2SO_4) to the caustic CIP solution can increase the rProtein A stability but might decrease the cleaning efficiency. Lower NaOH concentrations (10-30 mM) was not efficient for cleaning of MabSelect in this specific MAb process.

As alternative cleaning protocol using 6 M guanidine hydrochloride can be used, also in combination with reducing agent. To remove hydrophobically bound substances a solution of non-ionic detergent or ethanol is recommended.

Recommendations for sanitization of MabSelect Xtra (reducing microbial contamination of the packed bed to a minimum) with a number of well-proven methods are listed in Instruction 11-0026-02.

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Scale-up

After optimizing the antibody purification at laboratory-scale, the process can be scaled up to larger columns.

MabSelect Xtra has been successfully packed in large-scale columns in our laboratories (Fig 8). As noted in Table 1, at 30 cm or larger diameter scale (where wall effects are no longer a consideration), the maximum recommended operating velocity for MabSelect Xtra is 300 cm/h at 20 cm bed height with a low viscosity buffer.

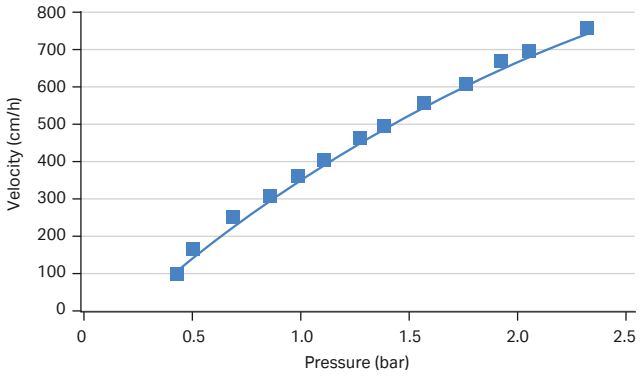


Fig 8. Typical pressure/flow curve obtained in AxiChrom 600, at 20 cm bed height. System/tubing pressure is included in the pressure.

Packing methods for large-scale columns

Good column packing is essential for any chromatographic process and naturally plays a key role in large-scale commercial manufacture. Proper packing will eliminate concerns such as channeling and compression and ensure a stable bed that performs according to expectations over many processing cycles.

At process scale, the preferred packing technique is axial compression. Using AxiChrom™ columns, with Intelligent Packing and preprogrammed packing methods for all MabSelect products, is an effective approach.

Test results show that MabSelect Xtra is both easy to pack and repack reproducibly at large scale.

Table 2 lists recommended large-scale column details.

Table 2. Recommended columns for MabSelect Xtra

Column family range	Inner diameter (mm)
Lab scale:	
Tricorn™	5, 10
HiScale™	16, 26, 50
Pilot and production scale:	
AxiChrom	50 – 1000
BPG™	100 – 300†
Chromaflow™	400 – 800‡

† The pressure rating of BPG 450 is too low to use it with MabSelect Xtra media.
‡ Larger pack stations might be required at larger diameters.

Methods for large-scale packing of MabSelect Xtra are available from Cytiva.

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Process economy

Lower cost of antibody capture

An important, if not the most important, consideration in bioprocessing is the economic aspect of the process. This is an especially important factor considering the anticipated increase in fermentation volumes and expression levels.

For a primary recovery step, the purification cost can be broken down into two types of contributions, one related to the number of cycles and the other related to the process time. In the first group, the cost of chemicals including chromatography media, as well as quality control are two important factors that need to be considered. In the second group, labour and facility costs may be predominant factors. The increase in dynamic binding capacity affects process economy because a lower number of cycles leads to a less frequent replacement of protein A media and to a proportionally lower buffer consumption.

For example, an increase of 30%, which should be the typical dynamic binding capacity gain of using MabSelect Xtra, results in about 25%–30% lower overall cost of the capture step (Fig 9).

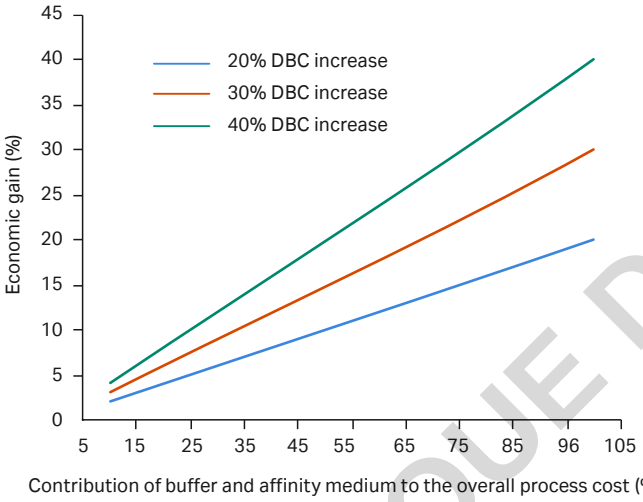


Fig 9. Effect of an increase in dynamic binding capacity on process economy. The economic gain is shown for a 20%, 30%, and 40% increase in DBC.

Application — purification of IgG_4

The capture of IgG_4 (pI 6.5–7.5) summarized below was performed on a FineLINE™ Pilot 35 column packed with MabSelect Xtra using an ÄKTApilot™ chromatography system.

The column was packed with MabSelect Xtra to a bed height of 20 cm and run at a nominal fluid velocity of 300 cm/h, which gave a residence time of 4 min. The purification cycle, which included CIP was repeated three times.

Figure 10 shows the running conditions and chromatogram of the first run. IgG_4 yield and purity were high over all three runs. Average yield was 94% and the monomer form of the material as determined by analytical gel filtration on Superdex™ 200 HR exceeded 99% (Fig 11).

The goal of achieving high recovery and product purity with the capture step run at pilot-scale on the FineLINE Pilot 35 column was clearly reached (the eluted antibody contained more than 99% monomer). In addition, the purification step was very consistent; the same separation and analytical results were found in all three runs. This work is further described in Application Note 11-0011-58. Pilot-scale capture of monoclonal antibody IgG_4 on MabSelect Xtra with high purity and yield.

Column:	FineLINE Pilot 35 (bed height 20.1 cm, 1 column volume =193 mL) packed with MabSelect Xtra
Sample:	IgG_4 clarified NSO supernatant,
Sample load:	5.16 L (0.55 g/L)
Buffer A:	10 mM sodium phosphate, 150 mM NaCl, pH 7.2
Buffer B:	100 mM glycine-HCl, pH 3.5
Flow rate:	300 cm/h (48 mL/min)
Gradient:	0%–100% B in 0 CV
CIP:	50 mM NaOH, 500 mM Na_2SO_4, contact time 10 min (46 mL/min)
System:	ÄKTApilot

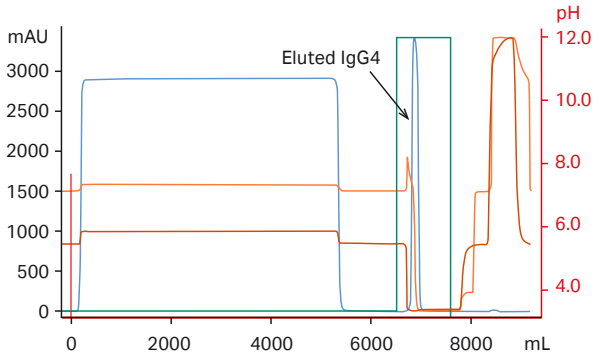


Fig 10. Capture of IgG_4 on MabSelect Xtra packed in a FineLINE Pilot 35 column. Average yield (94%) and purity (more than 99% monomer) were high over the three runs performed. The NSO cell line feedstock containing the IgG_4 monoclonal antibody was supplied by Lonza Biologics, UK.

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Column: Tricorn 10/300 (bed height 30 cm, 1 column volume = 23.56 mL)
Medium: Superdex 200 HR
Sample: IgG_4 purified on MabSelect Xtra from clarified NSO supernatant, Lonza Biologics
Sample volume: 50 µL
Buffer A: PBS, pH 7.4
Flow rate: 39 cm/h (0.5 mL/min)
System: ÄKTAexplorer™ 100

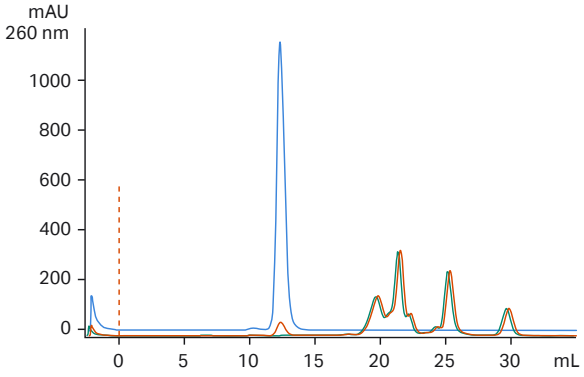


Fig 11. Dimer/aggregate analyses on Superdex 200 HR of the start material showing clarified IgG_4 supernatant (orange), flowthrough (green), and eluate after the capture step (blue). Note that no monomer of IgG_4 was found in the flowthrough (green line).

References

1. Grönberg, A. *et al.,* Cytiva. Poster at 1st High-Throughput Process Development International Conference, Krakow, Poland (2010).

Ordering information

Product	Pack Size	Code No.
MabSelect Xtra	25 mL	17-5269-07
	200 mL	17-5269-02
	1 L	17-5269-03
	5 L	17-5269-04
	10 L	17-5269-05
	60 L	17-5269-06
HiScreen MabSelect Xtra	1 × 4.7 mL	28-9269-76
HiTrap MabSelect Xtra	5 × 1 mL	28-4082-58
	5 × 5 mL	28-4082-61
PreDictor MabSelect Xtra	6 µL (4 × 96-well plates)	28-9432-75
	20 µL (4 × 96-well plates)	28-9432-76
	50 µL (4 × 96-well plates)	28-9432-77
PreDictor RoboColumn™ MabSelect Xtra	50 µL (one row of 8 columns)	28-9862-04
	200 µL (one row of 8 columns)	28-9861-08

Related literature

Data files	Code No.
MabSelect	18-1149-94
MabSelect SuRe	11-0011-65
MabSelect SuRe LX	28-9870-62
PreDictor 96-well filter plates and Assist software	28-9258-39
PreDictor RoboColumn	28-9886-34
HiTrap MabSelect, MabSelect Xtra, MabSelect SuRe	11-0034-90
HiScreen prepacked columns	28-9305-81
BPG columns	18-1115-23
HiScale columns	28-9755-23
AxiChrom columns	28-9290-41
Chromaflow columns	18-1138-92

Application notes	
MabSelect Xtra – IgG_4 purification on ÄKTApilot	11-0011-58

Handbooks	
Antibody purification, principles and methods	18-1037-46
High-throughput process development with PreDictor plates, principles and methods	28-9403-58

All bulk media products are supplied in suspension in 20% ethanol. For additional information, please contact your local Cytiva representative.

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