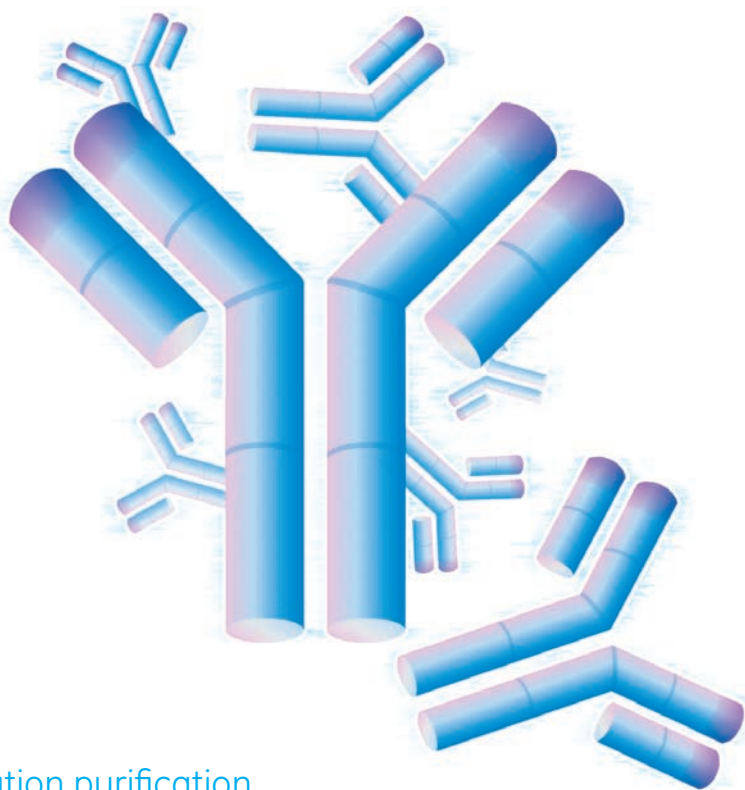


GE Healthcare

# Solutions for antibody purification

Selection Guide



High-resolution purification  
Batch/gravity-flow purification  
Small-scale purification  
High-throughput screening  
Process development



 Sepharose

# Small-scale purification

## Protein G chromatography media

Which species of antibodies will you primarily purify? See Table 1 "Relative Binding Strengths" for guidance on which ligand to choose.

**Protein G Sepharose**

Will you use an automated purification system such as ÄKTAdesign? No

Yes

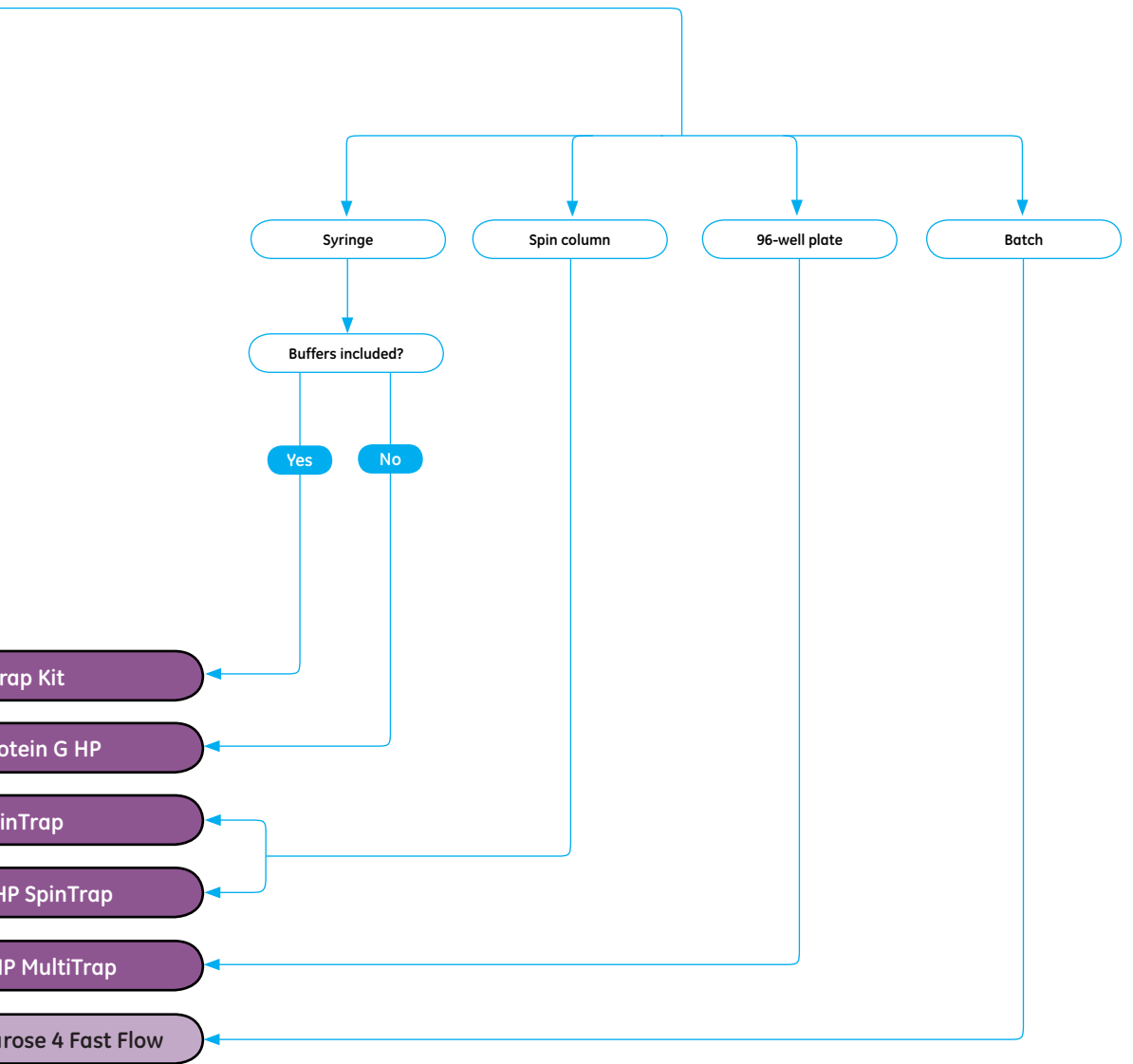
What is most important to you?

Scalability

High resolution

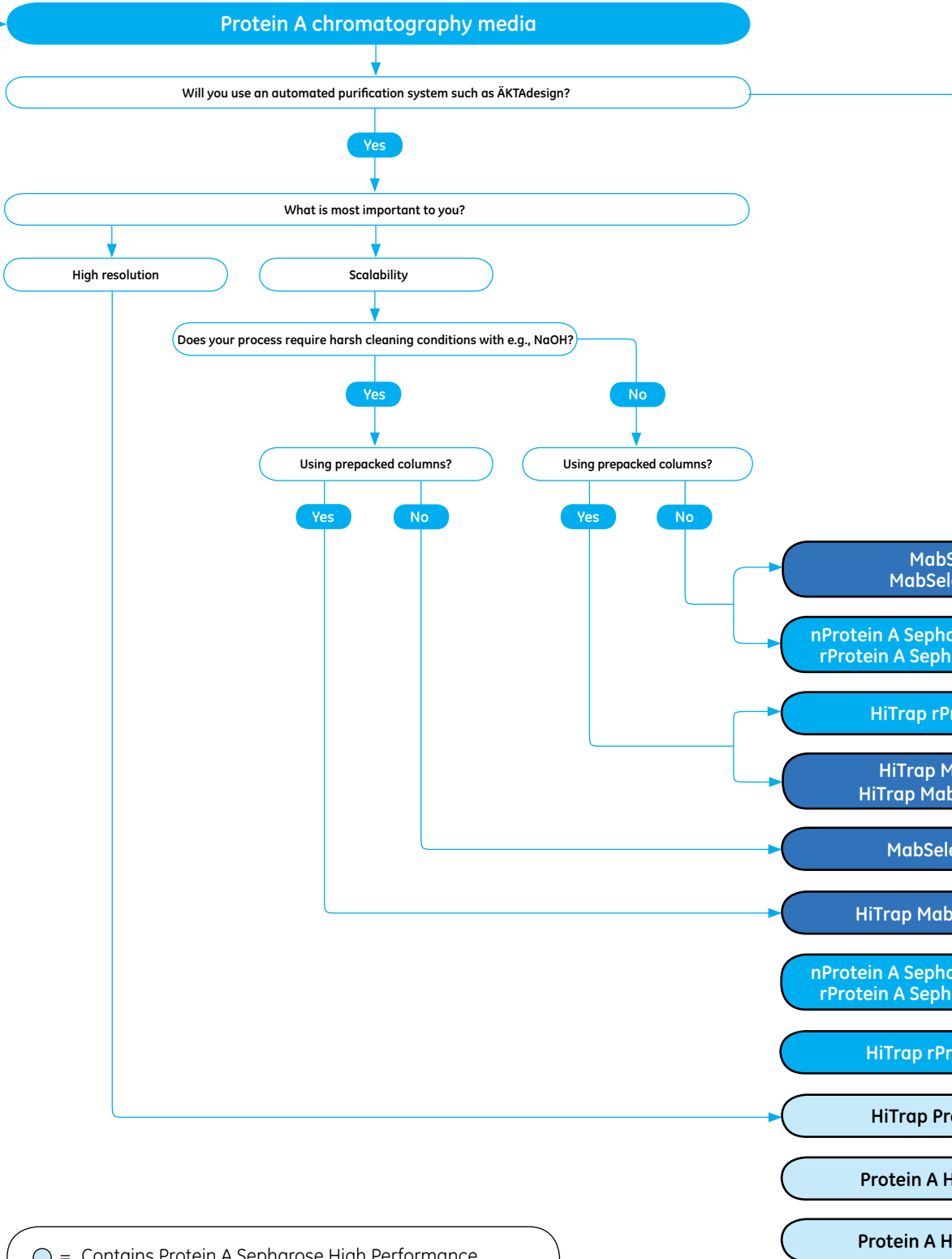
- MAbT
- HiTrap Pro
- Ab Sp
- Protein G H
- Protein G H
- Protein G Sepha





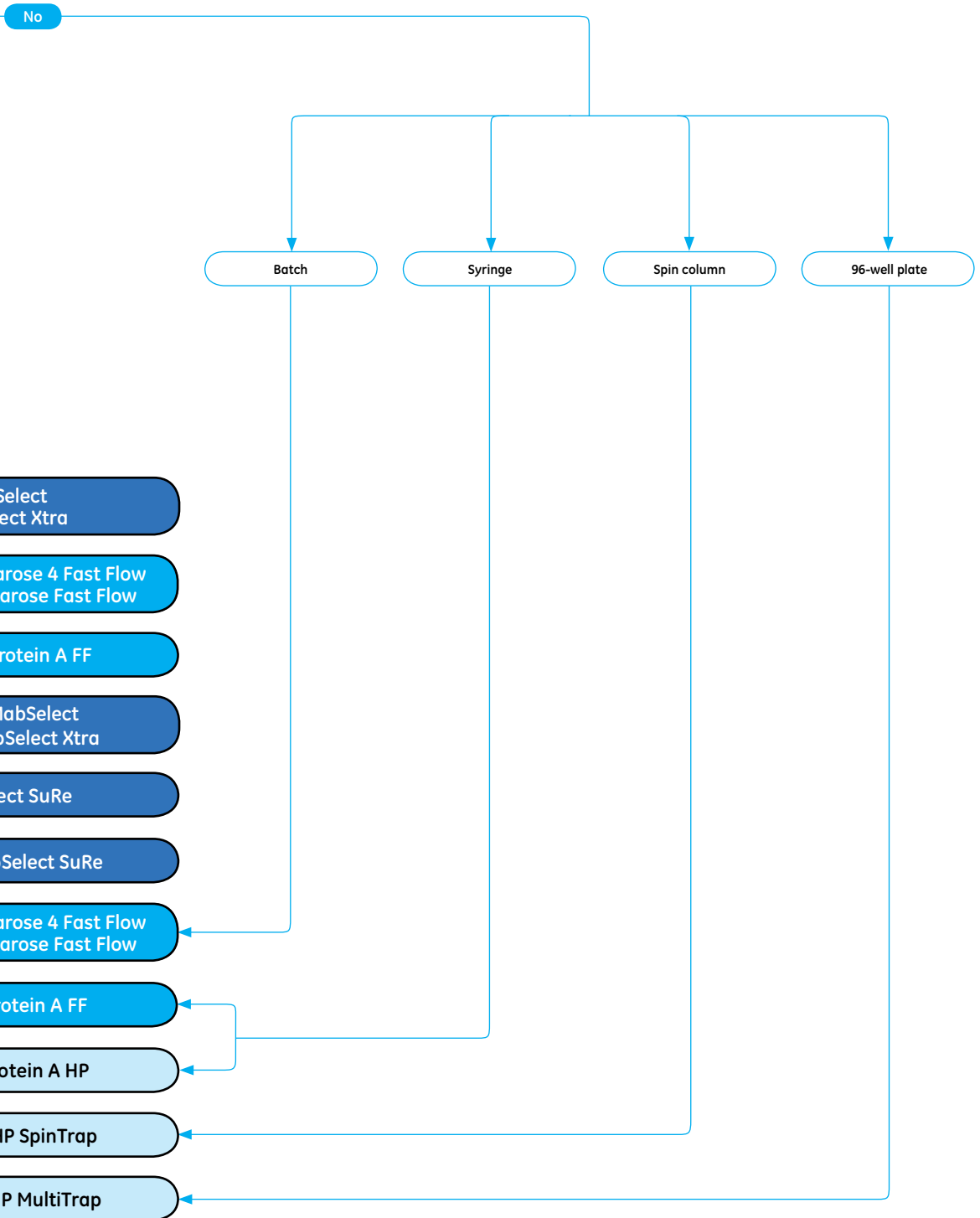
- = Contains Protein G Sepharose High Performance
- = Contains Protein G Sepharose 4 Fast Flow

# Protein A chromatography media



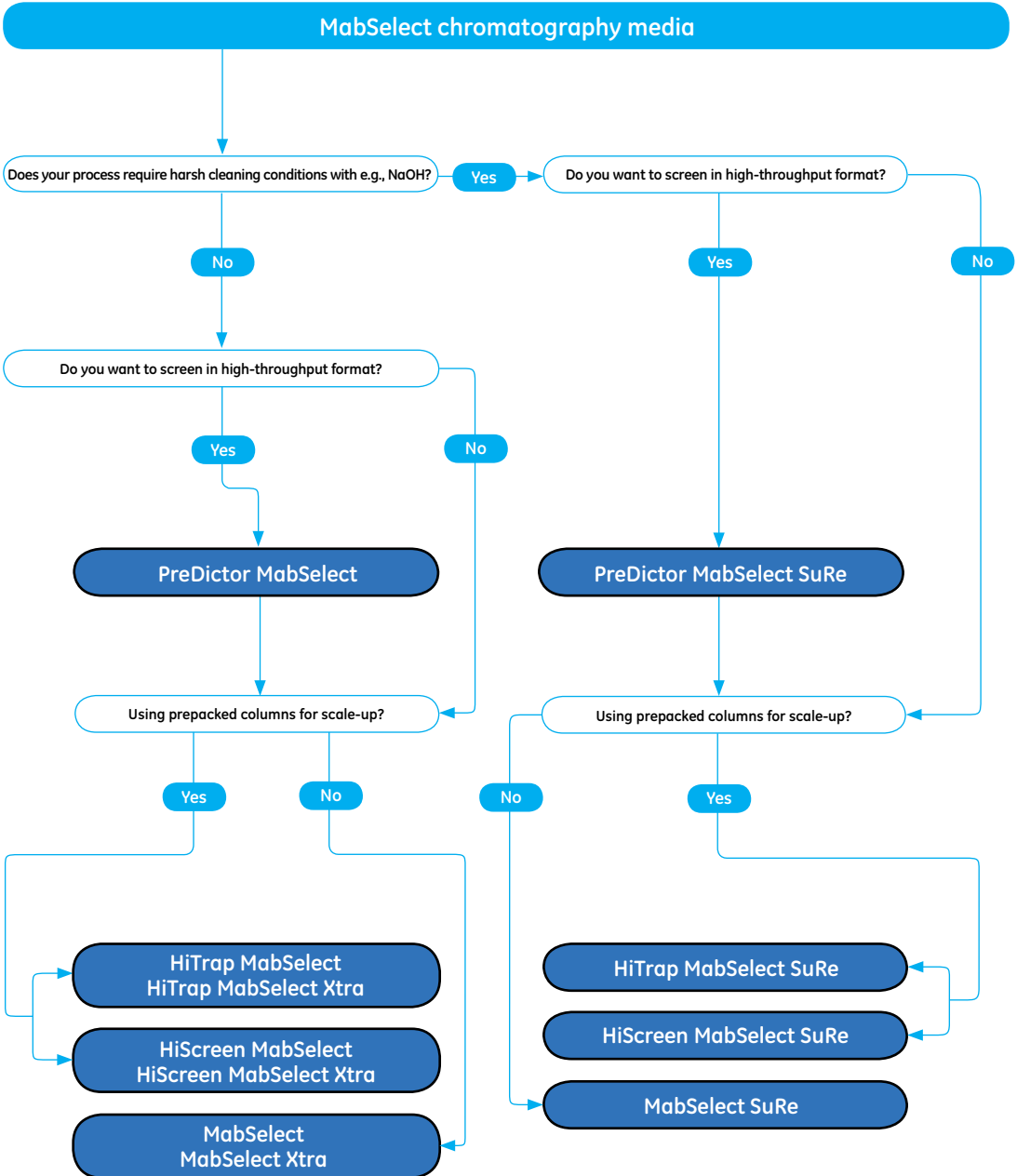
- = Contains Protein A Sepharose High Performance
- = Contains nProtein A or rProtein A Sepharose 4 Fast Flow
- = MabSelect media family

- MabSelect Media
- nProtein A Sepharose 4 Fast Flow  
rProtein A Sepharose 4 Fast Flow
- HiTrap rProtein A Sepharose 4 Fast Flow
- HiTrap MabSelect Media
- MabSelect Media
- HiTrap MabSelect Media
- nProtein A Sepharose 4 Fast Flow  
rProtein A Sepharose 4 Fast Flow
- HiTrap rProtein A Sepharose 4 Fast Flow
- HiTrap Protein A HPLC
- Protein A HPLC
- Protein A HPLC



# Process development

## MabSelect chromatography media family



● = MabSelect media family

*PreDicator MabSelect Xtra will be available beginning of 2009.*

# General information

The basis for antibody affinity purification is the high affinity and specificity of protein G and protein A for the Fc region of IgG from a variety of species. Protein G and protein A have been immobilized to several different matrices resulting in an excellent means of isolating IgG and IgG subclasses from ascites, cell culture supernatants, and serum.

Protein G and protein A are bacterial proteins from Group G *Streptococci* and *Staphylococcus aureus*, respectively. When coupled to Sepharose base matrices, protein G and protein A create useful, easy-to-use media for routine purification of antibodies.

GE Healthcare offers a recombinant form of protein G grown in *E. coli* from which the albumin-binding region of the native protein has been genetically deleted. This recombinant protein G ligand is coupled to both Sepharose 4 Fast Flow and Sepharose High Performance.

Native protein A (nProtein A) is also available coupled to Sepharose 4 Fast Flow and Sepharose High Performance while recombinant protein A (rProtein A) coupled to Sepharose Fast Flow is also offered. The MabSelect family of media also utilizes recombinant variants of protein A as ligand.

Protein G is a good first choice for general purpose capture of antibodies at laboratory scale since it binds a broader range of IgG from eukaryotic species and also binds to more subclasses of IgG, see Table 1. Usually, protein G has greater affinity for IgG than protein A and exhibits minimal binding to albumin, resulting in cleaner preparations and greater yields. The binding strength of protein G for IgG depends on the source species and subclass of the immunoglobulin. The dynamic binding capacity depends on the binding strength and also on several other factors, such as flow rate during sample application.

**Table 1.** Relative binding strengths of antibodies from various species to protein G and protein A as measured in a competitive ELISA test. The amount of IgG required to give a 50% inhibition of binding of rabbit IgG conjugated with alkaline phosphatase was determined

Species	Subclass	Protein G binding	Protein A binding
Human	IgA	—	variable
	IgD	—	—
	IgE	—	—
	IgG <sub>1</sub>	++++	++++
	IgG <sub>2</sub>	++++	++++
	IgG <sub>3</sub>	++++	—
	IgG <sub>4</sub>	++++	++++
	IgM	—	variable
Avian egg yolk	IgY	—	—
Cow		++++	++
Dog		+	++
Goat		++	—
Guinea pig	IgG <sub>1</sub>	++	++++
Hamster		++	+
Horse		++++	++
Koala		+	—
Llama		+	—
Monkey (rhesus)		++++	++++
Mouse	IgG <sub>1</sub>	++++	+
	IgG <sub>2a</sub>	++++	++++
	IgG <sub>2b</sub>	+++	+++
	IgG <sub>3</sub>	+++	++
	IgM	—	variable
Pig		+++	+++
Rabbit		+++	++++
Rat	IgG <sub>1</sub>	+	—
	IgG <sub>2a</sub>	++++	—
	IgG <sub>2b</sub>	++	—
	IgG <sub>3</sub>	++	+
Sheep		++	+/-

++++ = strong binding

++ = medium binding

— = weak or no binding

# Protein G Sepharose products

## HiTrap Protein G HP for convenient high-resolution purification

HiTrap™ Protein G HP 1 ml and 5 ml columns are ready-to-use columns for rapid, high-resolution laboratory-scale antibody purification with a syringe, pump, or chromatography system such as ÄKTAdesign™. The columns are prepacked with Protein G Sepharose™ High Performance, which has high binding capacity for antibodies. The small bead size (34 µm) ensures narrow elution peaks containing concentrated material.

## Ab SpinTrap columns for small-scale antibody purification

Ab SpinTrap™\* are prepacked, single-use spin columns for minipreps of monoclonal and polyclonal antibodies from unclarified serum and cell culture supernatants. The columns are designed for small-scale purification of multiple samples in parallel and are suitable for use in antibody screening experiments. Ab SpinTrap contains 100 µl of Protein G Sepharose High Performance. The columns are used together with a standard microcentrifuge and one purification run takes less than 20 min.

## Protein G HP SpinTrap for protein enrichment

Protein G HP SpinTrap\* are prepacked with 100 µl of Protein G Sepharose High Performance. The columns are single-use spin columns for protein enrichment of target antigens from antibody-antigen complexes of monoclonal and polyclonal antibodies from unclarified serum and cell culture supernatants (immuno-precipitation or pull-down technique). In addition, the columns are designed for small-scale purification of multiple samples in parallel and are suitable for use in antibody screening experiments. The columns are used together with a standard microcentrifuge.

## Protein G HP MultiTrap 96-well plates for high-throughput screening

Protein G HP MultiTrap™ 96-well plates are prepacked with Protein G Sepharose High Performance. Protein G HP MultiTrap is a versatile tool for screening of different proteins and for preparation of protein samples, enrichment of proteins of interest from clarified cell lysates and biological fluids, and small-scale purification of antibodies. Purification runs are performed in parallel, which ensures fast and reliable capture of antibodies from numerous complex samples.

## MABTrap Kit- easy purification and fast results

MABTrap™ Kit contains one HiTrap Protein G HP 1 ml column, binding, elution, and neutralization buffers, a syringe with fittings, and an optimized purification protocol.

The kit contains sufficient material for up to 20 purifications of monoclonal or polyclonal IgG from serum, cell culture supernatant, or ascites, when using a syringe. This kit is an excellent tool when no pump is available.

## Protein G Sepharose 4 Fast Flow for batch/gravity purification and scale-up

Protein G Sepharose 4 Fast Flow consists of 90 µm beads of highly cross-linked agarose, which provide a robust and stable chromatography matrix. The medium is a good choice for general-purpose capture of antibodies and scale-up in the laboratory.

\* Protocols for both small-scale antibody purification and enrichment of protein of interest with immunoaffinity are available





# Protein A Sepharose products

## HiTrap Protein A HP for convenient high-resolution purification

HiTrap Protein A HP 1 ml and 5 ml columns are ready-to-use columns for rapid, high-resolution antibody purification with a syringe, pump, or chromatography system such as ÄKTAdesign. The columns are prepacked with Protein A Sepharose High Performance, which has high binding capacity for antibodies. The small bead size (34 µm) ensures the lowest sample dilution and best high-resolution separation.

## Protein A HP SpinTrap for protein enrichment

Protein A HP SpinTrap\* are prepacked with 100 µl Protein A High Performance. The columns are single-use spin columns for protein enrichment of target antigens from antibody-antigen complexes of monoclonal and polyclonal antibodies from unclarified serum and cell culture supernatants (immunoprecipitation or pull-down technique). In addition, the columns are designed for small-scale purification of multiple samples in parallel and are suitable for use in antibody screening experiments. The columns are used together with a standard microcentrifuge.

## Protein A HP MultiTrap 96-well plates for high-throughput screening

Protein A HP MultiTrap 96-well plates are prepacked with Protein A Sepharose High Performance. Protein A HP MultiTrap is a versatile tool for screening of different proteins and for preparation of protein samples, enrichment of proteins of interest from clarified cell lysates and biological fluids, and small-scale purification of antibodies. Purification runs are performed in parallel, which ensures fast and reliable capture of antibodies from numerous complex samples.

## rProtein A Sepharose Fast Flow-enhanced binding capacity medium for batch/gravity purification and scale-up

rProtein A Sepharose Fast Flow consists of 90 µm beads of highly cross-linked agarose, which provide a robust and stable chromatography matrix. The medium is an excellent choice for batch purification/protein preps and scale-up. The recombinant protein A ligand of rProtein A Sepharose Fast Flow has been specially engineered to favor an oriented coupling giving a matrix with enhanced binding capacity for monoclonal and polyclonal antibodies compared to nProtein A Sepharose 4 Fast Flow.

## HiTrap rProtein A FF for fast and convenient purification

HiTrap rProtein A FF 1 ml and 5 ml are ready-to-use columns prepacked with rProtein A Sepharose Fast Flow for the convenient purification of monoclonal antibodies from cell culture supernatants, serum, and ascites. The column is suitable for small-scale purification of monoclonal antibodies from multiple species.

## nProtein A Sepharose 4 Fast Flow for batch/gravity purification and scale-up

nProtein A Sepharose 4 Fast Flow is native protein A coupled to Sepharose 4 Fast Flow. The medium is designed for recovery and purification of polyclonal and monoclonal antibodies from cell culture supernatants, serum, and ascites at both laboratory and larger scale.

\* Protocols for both small-scale antibody purification and enrichment of protein of interest with immunoaffinity are available



# MabSelect products

MabSelect™ chromatography media are BioProcess™ affinity media for capture of monoclonal antibodies (MAbs) from large volumes of feed by packed bed chromatography. The low ligand leakage of the ligand combined with the stability of the novel base matrix make MabSelect chromatography media suitable for purification of MAbs at process scale.

## MabSelect for high-throughput capture of MAbs

MabSelect consists of 85 µm beads of a highly cross-linked agarose base matrix. The recombinant protein A ligand of MabSelect is engineered to favor an oriented coupling that delivers enhanced binding capacity.

## MabSelect Xtra for capture of high-titer feedstreams

MabSelect Xtra™ uses the same recombinant protein A ligand as MabSelect, but the medium has a smaller particle size (75 µm) and greater porosity for increased dynamic binding capacity at high flow rates.

## MabSelect SuRe - designed to tolerate harsh cleaning-in-place protocols

MabSelect SuRe™ has been developed from the same highly cross-linked agarose matrix used for MabSelect, which enables high flow rates at low backpressure. In contrast to the recombinant protein A ligand of MabSelect, the alkali-tolerant recombinant protein A ligand of MabSelect SuRe is resistant to harsh cleaning agents (e.g., 0.1 to 0.5 M NaOH), which facilitates cleaning-in-place and results in significant cost savings. The high alkaline tolerance of the medium allows an increased number of cleaning-in-place cycles in regular large-scale production.

## PreDicator MabSelect products for high-throughput process development

PreDicator™ MabSelect and PreDicator MabSelect SuRe are 96-well filter plates prefilled with MabSelect and MabSelect SuRe, respectively. PreDicator plates support high-throughput process development (HTPD) by enabling parallel screening of chromatographic conditions, either in manual or in automated workflows. Data generated using PreDicator plates correlate well with data obtained in chromatography columns, making the plates an excellent tool for initial screening of process conditions. The 96-well plates are available in packs containing different chromatography media volumes to allow use in different applications. Once optimal process conditions have been found, these can be verified and fine-tuned in packed-bed columns.

## HiTrap MabSelect prepacked columns for convenient, small-scale purification

These HiTrap 1 ml and 5 ml columns are ready-to-use columns prepacked with all three different MabSelect chromatography media for small-scale purification and use in process development.

## HiScreen MabSelect prepacked columns for optimization of chromatography conditions

Prepacked ready-to-use columns with the three different MabSelect media for convenient optimization of chromatography conditions in process development. The 10 cm bed height of HiScreen™ columns enables effective parameter screening and method optimization. Once optimal process conditions have been defined, the chromatography process can be scaled up to larger columns for process-scale antibody production.



# Products for antibody purification

Product	Product Code No.	Pack size						Data file Code No.	Approx. protein binding capacity
			High throughput screening	Mimipreps	Batch/gravity flow	Syringe compatible	Prepacked columns		
HiTrap Protein G HP	17-0404-01	5 × 1 ml				•	•	11-0035-58	25 mg/column
	17-0404-03	2 × 1 ml				•	•		
	17-0405-01	1 × 5 ml				•	•	11-0035-58	125 mg/column
	17-0405-03	5 × 5 ml				•	•		
Ab SpinTrap	28-4083-47	50 × 100 µl	•					28-9020-30	1 mg/column
Protein G HP SpinTrap	28-9031-34	16 × 100 µl	•					28-9067-90	1 mg/column
Protein G HP MultiTrap	28-9031-35	4 × 96 well plates	•					28-9067-90	0.5 mg/well
MABTrap Kit*	17-1128-01	1 × 1 ml				•		18-1034-14	25 mg/column
Protein G Sepharose 4 Fast Flow	17-0618-01	5 ml	•	•	•			18-1012-91	20 mg/ml
	17-0618-02	25 ml	•	•	•				
HiTrap Protein A HP	17-0402-01	5 × 1 ml				•	•	11-0035-58	20 mg/column
	17-0402-03	2 × 1 ml				•	•		
	17-0403-01	1 × 5 ml				•	•	11-0035-58	100 mg/column
	17-0403-03	5 × 5 ml				•	•		
Protein A HP SpinTrap	28-9031-32	16 × 100 µl	•					28-9067-89	1 mg/column
Protein A HP MultiTrap	28-9031-33	4 × 96 well plates	•					28-9067-89	0.5 mg/well
rProtein A Sepharose Fast Flow	17-1279-01	5 ml	•	•	•			18-1113-94	50 mg/ml
	17-1279-02	25 ml	•	•	•				
HiTrap rProtein A FF	17-5079-01	5 × 1 ml				•	•	11-0035-58	50 mg/column
	17-5079-02	2 × 1 ml				•	•		
	17-5080-01	1 × 5 ml				•	•	11-0035-58	250 mg/column
	17-5080-02	5 × 5 ml				•	•		
nProtein A Sepharose 4 Fast Flow	17-5280-01	5 ml	•	•	•			18-1125-19	30 mg/ml
	17-5280-04	25 ml	•	•	•				
MabSelect	17-5199-01	25 ml					•	18-1149-94	30 mg/ml
HiTrap MabSelect	28-4082-53	5 × 1 ml				•	•	11-0034-90	30 mg/column
	28-4082-55	1 × 5 ml				•	•		
	28-4082-56	5 × 5 ml				•	•		
HiScreen MabSelect	28-9269-73	1 × 4.7 ml				•	•	28-9305-81	140 mg/column
PreDictor MabSelect, 6 µl	28-9258-20	4 × 96 well plates	•				•	28-9258-39	N/A <sup>†</sup>
PreDictor MabSelect, 20 µl	28-9258-21	4 × 96 well plates	•				•		N/A <sup>†</sup>
PreDictor MabSelect, 50 µl	28-9258-22	4 × 96 well plates	•				•		N/A <sup>†</sup>
MabSelect SuRe	17-5438-01	25 ml					•	11-0011-65	30 mg/ml
HiTrap MabSelect SuRe	11-0034-93	5 × 1 ml				•	•	11-0034-90	30 mg/column
	11-0034-94	1 × 5 ml				•	•		
	11-0034-95	5 × 5 ml				•	•		
HiScreen MabSelect SuRe	28-9269-77	1 × 4.7 ml				•	•	28-9305-81	140 mg/column
PreDictor MabSelect SuRe, 6 µl	28-9258-23	4 × 96 well plates	•				•	28-9258-39	N/A <sup>†</sup>
PreDictor MabSelect SuRe, 20 µl	28-9258-24	4 × 96 well plates	•				•		N/A <sup>†</sup>
PreDictor MabSelect SuRe, 50 µl	28-9258-25	4 × 96 well plates	•				•		N/A <sup>†</sup>
MabSelect Xtra	17-5269-07	25 ml					•	11-0011-57	40 mg/ml
HiTrap MabSelect Xtra	28-4082-58	5 × 1 ml				•	•	11-0034-90	40 mg/column
	28-4082-60	1 × 5 ml				•	•		
	28-4082-61	5 × 5 ml				•	•		
HiScreen MabSelect Xtra	28-9269-76	1 × 4.7 ml				•	•	28-9305-81	188 mg/column

\* Includes buffer stock solution solutions for approximately 20 purifications using a syringe

<sup>†</sup> The products are used for high-throughput screening of chromatographic conditions (i.e., capacity, selectivity, purity)

For contact information for your local office,  
please visit, [www.gelifesciences.com/contact](http://www.gelifesciences.com/contact)

[www.gelifesciences.com/protein-purification](http://www.gelifesciences.com/protein-purification)

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