



Columns and resins for antibody purification and immunoprecipitation

Selection guide

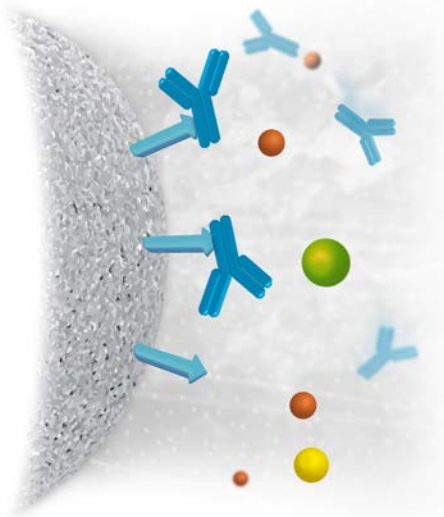


Introduction to antibody purification and immunoprecipitation

What is antibody purification?

Polyclonal antibodies, monoclonal antibodies (mAb), and antibody fragments are usually purified by affinity chromatography. Resins containing an immobilized ligand (e.g., protein A, protein G, or protein L) are used to capture antibodies and antibody fragments.

Affinity purification offers high selectivity. Purity levels above 95% are often possible in just one step.



How does antibody purification work?

Antibody affinity chromatography is based on the high affinity and specificity to affinity ligands.

The binding of an antibody to the ligand is reversible, and the antibody is often eluted by lowering the pH.

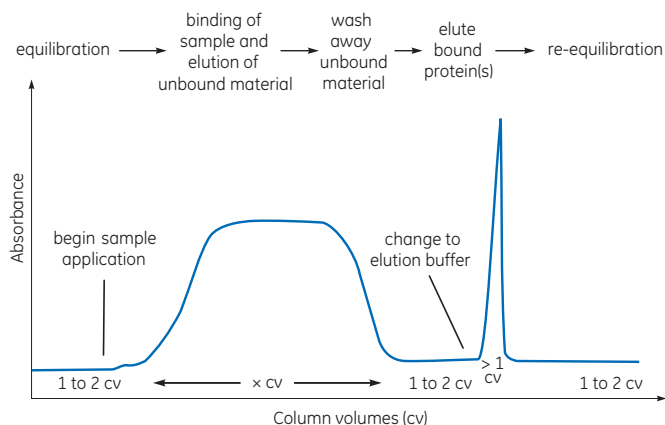


Fig 1. Typical affinity purification.

Affinity ligands for antibody purification

Protein A and protein G

While protein A and protein G affinity resins are similar in many respects, their specificities for immunoglobulin G (IgG) differ.

Protein G affinity resin 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 than Protein A (see Table 1).

The protein A ligand is routinely used in pharmaceutical processes and is commonly preferred when purifying human monoclonal antibodies.

Protein L

Protein L binds to the variable region of the kappa light chain. Therefore, protein L affinity resin is suitable for capture of a wide range of antibody fragments.

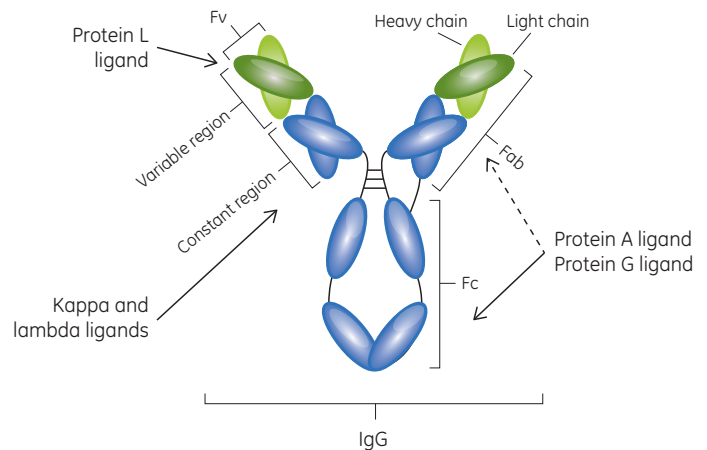


Fig 2. IgG, which is by far the most common immunoglobulin, is commonly purified with protein G and protein A, both of which have a strong affinity to the Fc region of IgG. Protein L has a strong affinity to the variable region of kappa light chains.

What is immunoprecipitation?

Immunoprecipitation (IP) is a highly specific and effective technique for analytical separations of target antigens from crude cell lysates. When combined with other techniques, such as SDS-PAGE and immunoblotting, IP can be used to analyze and quantify your antigen.

Which ligand should be used?

Binding affinities to affinity ligands

Table 1. Relative binding strengths of antibodies from various species to protein A, protein G, and protein L

Species	Antibody class	Affinity*		
		Protein A	Protein G	Protein L
Human	IgG ₁	+++	+++	+++
	IgG ₂	+++	+++	+++
	IgG ₃	-	+++	+++
	IgG ₄	+++	+++	+++
	IgA	Variable	-	+++
	IgD	-	-	+++
	IgE	-	-	+++
	IgM**	Variable	-	+++
Mouse	IgG ₁	+	+++	+++
	IgG _{2a}	+++	+++	+++
	IgG _{2b}	+++	+++	+++
	IgG ₃	+	+++	+++
	IgM**	Variable	-	+++
Rat	IgG ₁	-	+	+++
	IgG _{2a}	-	+++	+++
	IgG _{2b}	-	+	+++
	IgG _{2c}	nd	nd	+++
	IgG ₃	+	+	nd
Pig	Total IgG	+++	+++	+++
Dog	Total IgG	+	+	+
Cow	Total IgG	+	+++	-
Goat	Total IgG	-	+	-
Sheep	Total IgG	+/-	+	-
Chicken	Total IgG	nd	nd	-
Rabbit	Total IgG	+++	+++	nd
Avian egg yolk	IgY***	-	-	nd
Guinea pig	IgG ₁	+++	+	nd
Hamster	Total IgG	+	+	nd
Horse	Total IgG	+	+++	nd
Koala	Total IgG	-	+	nd
Llama	Total IgG	-	+	nd
Monkey (rhesus)	Total IgG	+++	+++	nd
Other	Kappa light chain (subtypes 1,3,4)	nd	nd	+++
	Lambda light chain	nd	nd	-
	Heavy chain	nd	nd	-
	Fab	+/-	+/-	+++
	ScFv	nd	nd	+++
	Dab	nd	nd	+++

+++ = strong binding

+ = weak binding

- = no binding

+/- = weak binding in some cases

nd = no data available

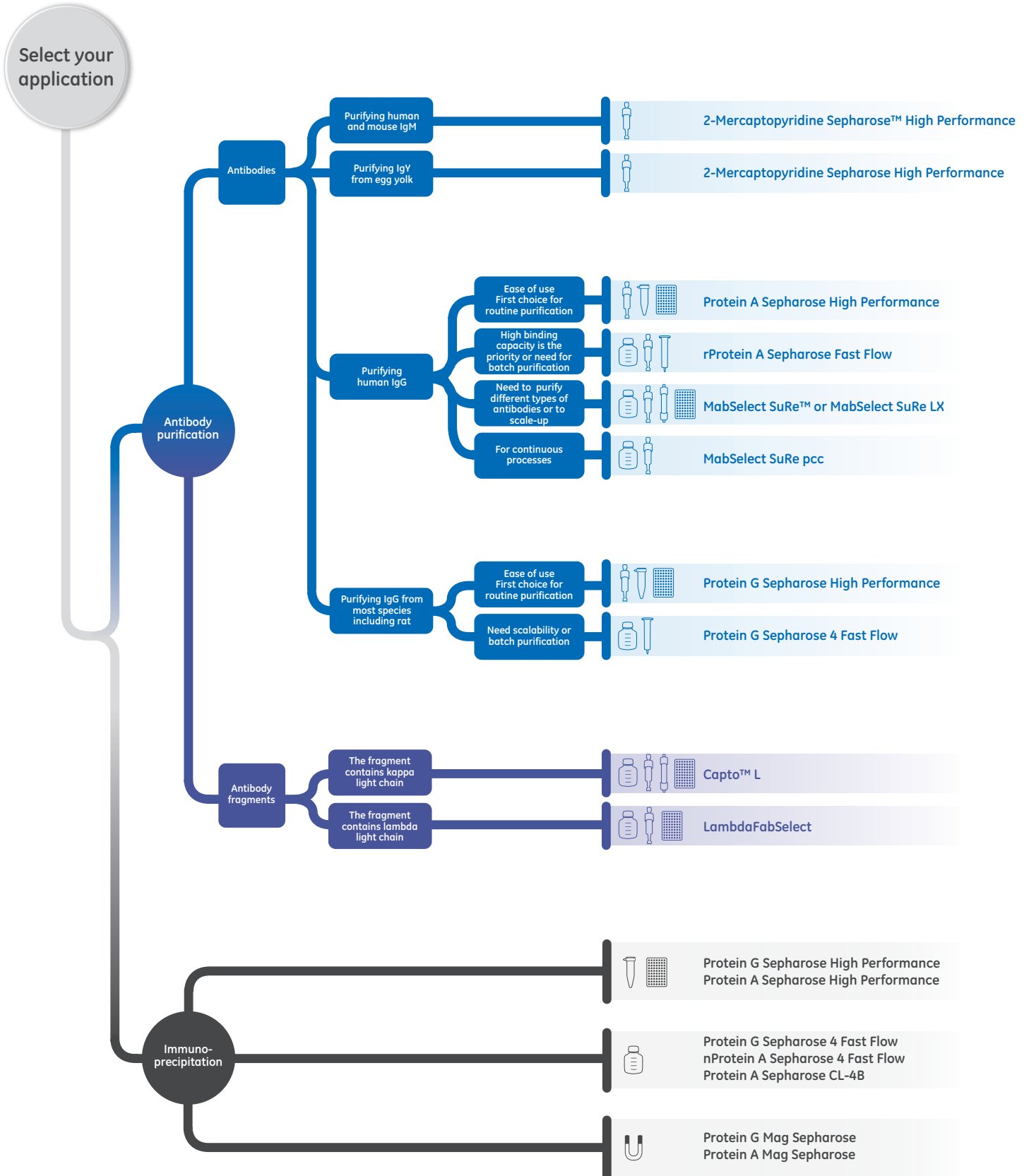
* Protein G and Protein A: Relative binding strengths of antibodies from various species to protein G and protein A as measured in a competitive ELISA test. The amount of IgG required to give a 50% inhibition of binding of rabbit IgG conjugated with alkaline phosphatase was determined.

Protein L: The binding of different radiolabeled IgGs to protein L-containing *Peptostreptococcus magnus* cells were measured. Relative binding strength of different IgGs to protein L is expressed as the percentage of bound IgG to the total amount of IgG. Binding to protein L occurs only if the immunoglobulin has the appropriate kappa light chains. Stated binding affinity refers only to species and subtypes with appropriate kappa light chains. Data from De Chateau, M. et al. On the interaction between protein L and immunoglobulins of various mammalian species. *Scand.J. Immunol.* 37, 399-405 (1993).

** Purified using HiTrap IgM Purification HP columns

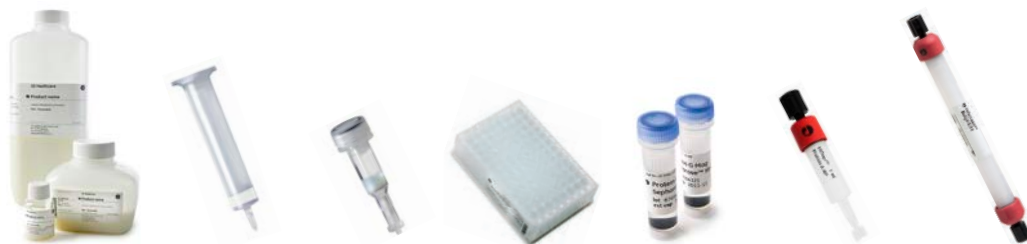
*** Purified using HiTrap IgY Purification HP columns

Select your antibody purification resin



Details on format icons are presented on next page.

Select the format according to your needs



	Resin in bulk	Gravity flow column (GraviTrap™)	Spin column (SpinTrap™)	96-well plate (MultiTrap™ and PreDicator™)	Magnetic beads (Mag Sepharose)	HiTrap™ column	HiScreen™ column
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Icon on page 4



Application

Small-scale preparative purification	✓	✓	✓	✓	✓	✓	✓
Process development	✓			✓		✓	✓
High-throughput screening	✓			✓	✓		
Immunoprecipitation (IP)	✓		✓	✓	✓		

Use

Batch adsorption	✓				✓		
Gravity	✓	✓					
Syringe						✓	
Peristaltic pump	✓					✓	
Centrifuge	✓		✓	✓			
Robotic system				✓	✓		
Chromatography system	All ÄKTA™ systems					ÄKTA pure 25 ÄKTA avant 25 ÄKTA start ÄKTAexpress ÄKTAprime plus ÄKTApurifier ¹ ÄKTAexplorer ² ÄKTA _{FPLC} ³	ÄKTA pure ÄKTA avant ÄKTA start ÄKTApurifier ¹ ÄKTAexplorer ²

¹ ÄKTApurifier has been discontinued and replaced by ÄKTA pure

² ÄKTAexplorer has been discontinued and replaced by ÄKTA avant

³ ÄKTA_{FPLC} has been discontinued and replaced by ÄKTA pure 25



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Ordering information

[Back to resin selection guide](#)

Protein A ligand-based resins

Resin	Main feature	Particle size, d_{50}^*	Format	Product name	Approx. binding capacity**	Volume	Pack size	Product code			
Protein A Sepharose High Performance (HP)	A good first choice for routine purifications. The small bead size (34 μm) ensures the lowest sample dilution of the eluted peak.	~ 34 μm	HiTrap column	HiTrap Protein A HP	20 mg/column	1 mL	1 column	29048576			
							2 columns	17040203			
							5 columns	17040201			
					100 mg/column	5 mL	1 column	17040301			
							5 columns	17040303			
					Spin column	Protein A HP SpinTrap	1 mg/column	100 μL	16 columns	28903132	
					96-well plate	Protein A HP MultiTrap	0.5 mg/well	100 μL /well	4 plates	28903133	
Protein A Sepharose CL-4B	A classic, well documented resin, suitable for immunoprecipitation procedures.	~ 90 μm	Resin in bulk	Protein A Sepharose CL-4B	20 mg/mL resin	25 mL	1 bottle	17096303			
						500 mL	1 bottle	17096302			
					20 mg/mL swelled resin	1.5 g	1 bottle	17078001			
rProtein A Sepharose Fast Flow (FF)	A good choice when high capacity or batch purification is needed. The recombinant protein A ligand has been engineered to favour an oriented coupling giving a matrix with enhanced binding capacity.	~ 90 μm	Resin in bulk	rProtein A Sepharose Fast Flow	50 mg/mL resin	5 mL	1 bottle	17127901			
						25 mL	1 bottle	17127902			
						200 mL	1 bottle	17127903			
			HiTrap column	HiTrap rProtein A FF	50 mg/column	1 mL	2 columns	17507902			
							5 columns	17507901			
			250 mg/column	5 mL	1 column	17508001					
					5 columns	17508002					
Gravity flow column	rProtein A GraviTrap	50 mg/column	1 mL	10 columns	28985254						
MabSelect SuRe	An excellent choice when purifying multiple types of antibodies, because the resin can be thoroughly cleaned between runs to minimize the risk for cross-contamination. The ligand is a recombinant alkali-tolerant protein A that is resistant to harsh cleaning agents (e.g., 0.1 to 0.5 M NaOH). Also recommended for scaling up.	~ 85 μm	Resin in bulk	MabSelect SuRe	30 mg/mL resin	25 mL	1 bottle	17543801			
						200 mL	1 bottle	17543802			
			HiTrap column	HiTrap MabSelect SuRe	30 mg/column	1 mL	1 column	29049104			
							5 columns	11003493			
					150 mg/column	5 mL	1 column	11003494			
			5 columns	11003495							
				HiScreen column	HiScreen MabSelect SuRe	141 mg/column	4.7 mL	1 column	28926977		
			96-well plate	PreDictor MabSelect SuRe, 6 μL	N/A [†]	6 μL /well	4 plates	28925823			
PreDictor MabSelect SuRe, 20 μL	N/A [†]	20 μL /well					4 plates	28925824			
PreDictor MabSelect SuRe, 50 μL	N/A [†]	50 μL /well					4 plates	28925825			
MabSelect SuRe LX	Same features as MabSelect SuRe with increased antibody binding capacity.	~ 85 μm	Resin in bulk	MabSelect SuRe LX	60 mg/mL resin	25 mL	1 bottle	17547401			
						200 mL	1 bottle	17547402			
			HiTrap column	HiTrap MabSelect SuRe LX	300 mg/column	5 mL	5 columns	29157185 [‡]			
			HiScreen column	HiScreen MabSelect SuRe LX	282 mg/column	4.7 mL	1 column	17547415			
			96-well plate	PreDictor MabSelect SuRe LX, 6 μL	N/A [†]	6 μL /well	4 plates	17547430			
							PreDictor MabSelect SuRe LX, 20 μL	N/A [†]	20 μL /well	4 plates	17547431
							PreDictor MabSelect SuRe LX, 50 μL	N/A [†]	50 μL /well	4 plates	17547432
MabSelect SuRe pcc	Offers exceptional capacity at high flow rates. Well suited for mAb capture in a continuous process. The ligand is a recombinant alkali-tolerant protein A that is resistant to harsh cleaning agents (e.g., 0.1 to 0.5 M NaOH).	~ 50 μm	Resin in bulk	MabSelect SuRe pcc	60 mg/mL resin	25 mL	1 bottle	17549101			
						200 mL	1 bottle	17549102			
			HiTrap column	HiTrap MabSelect SuRe pcc	60 mg/column	1 mL	5 columns	17549111			
					300 mg/column	5 mL	1 column	17549112			
Protein A Mag Sepharose	Magnetic beads designed to simplify enrichment of target proteins by immunoprecipitation. The magnetic beads are based on Sepharose with native protein A as ligand.	37 to 100 μm	Magnetic beads	Protein A Mag Sepharose	0.8 mg/vial (the vial contains 100 μL beads)	500 μL	1 vial	28944006			
							4 vials	28951378			

* Median particle size of the cumulative volume distribution.

** According to recommended protocols.

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

‡ This product is part of our Custom Designed Media program. Delivery time may be longer than for standard products.

Protein G ligand-based resins

Resin	Main feature	Particle size, d_{50}^{*} :	Format	Product name	Approx. binding capacity**	Volume	Pack size	Product code							
Protein G Sepharose HP	A good first choice for routine purifications. The small bead size (34 μm) ensures narrow elution of the eluted peak.	~ 34 μm	HiTrap column	HiTrap Protein G HP	25 mg/column	1 mL	1 column	29048581							
							2 columns	17040403							
							5 columns	17040401							
										125 mg/column	5 mL	1 column	17040501		
												5 columns	17040503		
										Spin column	Protein G HP SpinTrap	1 mg/column	100 μL	16 columns	28903134
											Ab SpinTrap	1 mg/column		50 columns	28408347
	96-well plate	Protein G HP MultiTrap	0.5 mg/well	100 μL /well	4 plates	28903135									
Protein G Sepharose 4 FF	A good choice when scaling up or batch purification is needed. Also suitable for immunoprecipitation procedures.	~ 90 μm	Resin in bulk	Protein G Sepharose 4 Fast Flow	20 mg/mL resin	5 mL	1 bottle	17061801							
							25 mL	1 bottle	17061802						
							200 mL	1 bottle	17061805						
							Gravity flow column	Protein G GraviTrap	20 mg/column	1 mL	10 columns	28985255			
Protein G Mag Sepharose	Magnetic beads designed to simplify enrichment of target proteins by immunoprecipitation. The magnetic beads are based on Sepharose with protein G as ligand.	37 to 100 μm	Magnetic beads	Protein G Mag Sepharose	1.3 mg/vial (the vial contains 100 μL beads)	500 μL	1 vial	28944008							
							4 vials	28951379							
Protein G Sepharose 4 FF nProtein A Sepharose 4 FF	A suitable starter kit for immunoprecipitation procedures, because it contains both protein A and protein G ligand-based resins.	~ 90 μm	Resin in bulk	Immunoprecipitation starter pack	Protein G Sepharose 4 FF: 20 mg/mL resin nProtein A Sepharose 4 FF: 20 mg/mL resin	2 mL Protein G Sepharose 4 Fast Flow and 2 mL nProtein A Sepharose 4 Fast Flow	2 bottles	17600235							
Protein G Sepharose 4 FF rProtein A Sepharose FF	See features above for each resin.	~ 90 μm	Gravity flow column	rProtein A/Protein G GraviTrap	35 mg/column	1 mL	10 columns	28985256							

IgM and IgY purification resins

Resin	Main feature	Particle size, d_{50}^{*} :	Format	Product name	Approx. binding capacity**	Volume	Pack size	Product code
2-Mercaptopyridine Sepharose HP	A thiophilic affinity resin designed for the purification of IgM, but it can also be used for purification of other immunoglobulins.	~ 34 μm	HiTrap column	HiTrap IgM Purification HP	5 mg IgM/column	1 mL	5 columns	17511001
2-Mercaptopyridine Sepharose HP	A thiophilic affinity resin designed for the purification of IgY, but it can also be used for purification of other immunoglobulins.	~ 34 μm	HiTrap column	HiTrap IgY Purification HP	100 mg IgY/column	5 mL	1 column	17511011

* Median particle size of the cumulative volume distribution.

** According to recommended protocols.

Handbook: Affinity Chromatography. Vol. 1 Antibodies

Looking for protocols and tips for using affinity chromatography for antibody purification? Download our handbook from gelifesciences.com/ProteinHandbooks





Antibody fragment purification resins

Resin	Main feature	Particle size, d_{50}^*	Format	Product name	Approx. binding capacity**	Volume	Pack size	Product code				
Capto L	The immunoglobulin-binding recombinant protein L ligand of Capto L has a strong affinity to the variable region of antibody kappa light chains. Capto L is the first choice for the capture of a wide range of antibody fragments.	~ 85 μ m	Resin in bulk	Capto L	25 mg human Fab/mL resin	5 mL	1 bottle	17547806				
						25 mL	1 bottle	17547801				
						200 mL	1 bottle	17547802				
			HiTrap column	HiTrap Protein L	25 mg human Fab/column	1 mL	1 column	29048665				
							5 columns	17547851				
						5 mL	1 column	17547815				
							5 columns	17547855				
							HiScreen column	HiScreen Capto L	118 mg human Fab/column	4.7 mL	1 column	17547814
						96-well plate	PreDictor Capto L, 6 μ L	N/A [†]	6 μ L	4 plates	17547830	
											PreDictor Capto L, 20 μ L	N/A [†]
PreDictor Capto L, 50 μ L	N/A [†]	50 μ L	4 plates	17547832								
LambdaFabSelect	An affinity resin designed for the purification of human Fab lambda fragments. The ligand binds to the constant region of the lambda light chain.	~ 75 μ m	Resin in bulk	LambdaFabSelect	20 mg polyclonal human Fab lambda/mL resin	25 mL	1 bottle	17548201 [‡]				
							200 mL	1 bottle	17548202 [‡]			
			HiTrap column	HiTrap LambdaFabSelect	20 mg polyclonal human Fab lambda/column	1 mL	5 columns	17548211 [‡]				
							100 mg polyclonal human Fab lambda/column	5 mL	1 column	17548212 [‡]		
						96-well plate	PreDictor LambdaFabSelect, 6 μ L	N/A [†]	6 μ L	4 plates	17548213 [‡]	
			PreDictor LambdaFabSelect, 20 μ L	N/A [†]	20 μ L						4 plates	17548214 [‡]
			PreDictor LambdaFabSelect, 50 μ L	N/A [†]	50 μ L						4 plates	17548215 [‡]
			KappaSelect	An affinity resin designed for the purification of human Fab kappa fragments. The ligand binds to the constant region of the kappa light chain. KappaSelect is a good second choice if Capto L does not work.	~75 μ m	Resin in bulk	KappaSelect	15 mg polyclonal human Fab kappa/mL resin	25 mL	1 bottle	17545801 [‡]	
										200 mL	1 bottle	17545802 [‡]
						HiTrap column	HiTrap KappaSelect	15 mg polyclonal human Fab kappa/column	1 mL	5 columns	17545811 [‡]	
45 mg polyclonal human Fab kappa/column	5 mL	1 column								17545812 [‡]		
96-well plate	PreDictor KappaSelect, 6 μ L	N/A [†]							6 μ L	4 plates	28980195 [‡]	
						PreDictor KappaSelect, 20 μ L	N/A [†]	20 μ L			4 plates	28980196 [‡]
						PreDictor KappaSelect, 100 μ L	N/A [†]	100 μ L			4 plates	28952733 [‡]

* Median particle size of the cumulative volume distribution.

** According to recommended protocols.

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[‡] This product is part of our Custom Designed Media program. Delivery time may be longer than for standard products.

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