Selection guide

Columns and resins for antibody purification and immunoprecipitation

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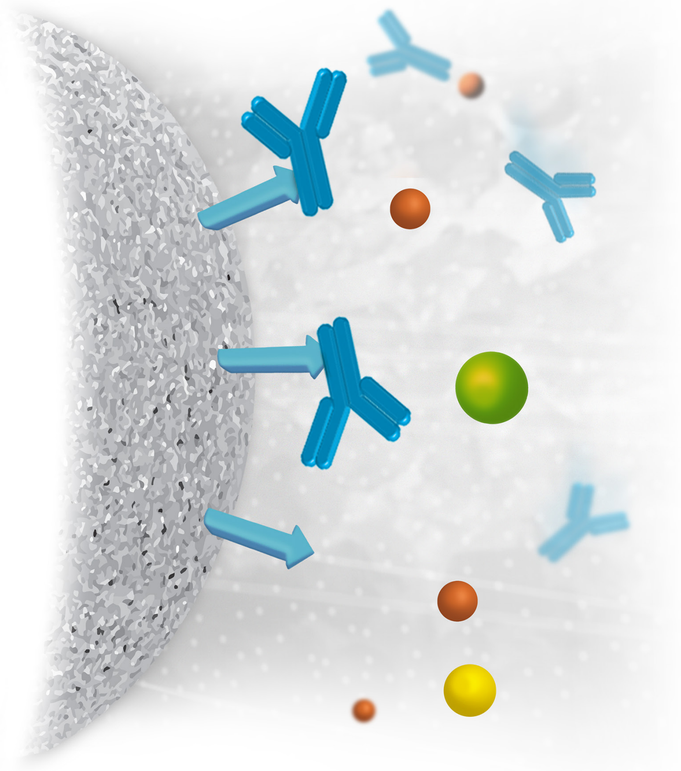
Introduction to antibody purification and immunoprecipitation

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.

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.

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.

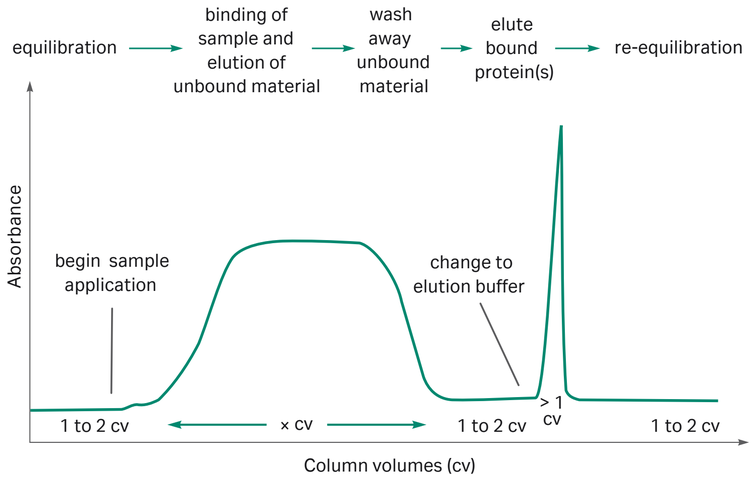


Fig 1. Typical affinity purification.

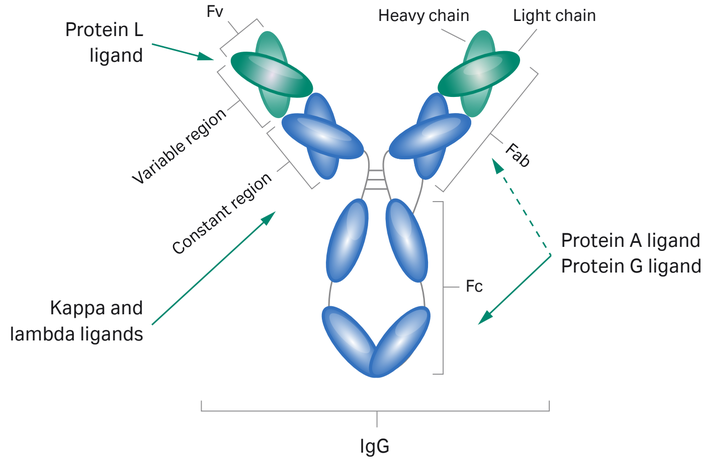


Fig 2. IgG, which is by far the most common immunoglobulin, is commonly purified with protein G and protein A, both of which have a strong affinity to the Fc region of IgG. Protein L has a strong affinity to the variable region of kappa light chains.

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

		Affinity*		
Species	**Antibody class**	**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

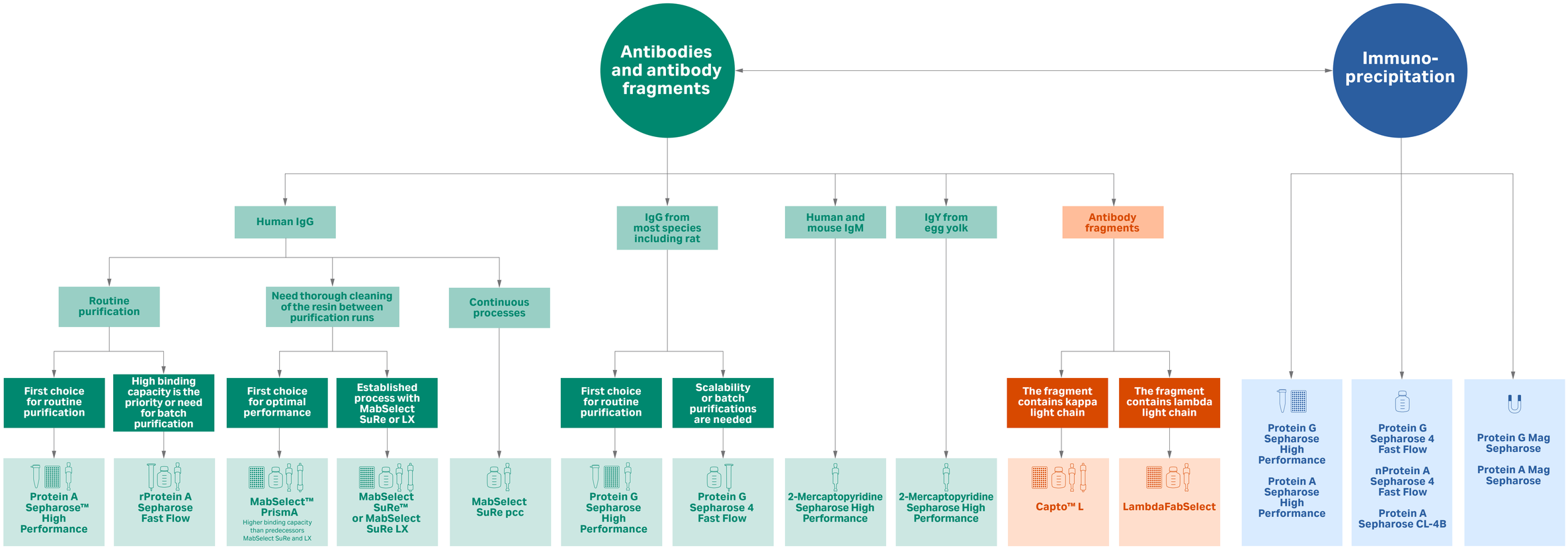
* Protein G and Protein A: Relative binding strengths of antibodies from various species to protein G and protein A as measured in a competitive ELISA test. The amount of IgG required to give a 50% inhibition of binding of rabbit IgG conjugated with alkaline phosphatase was determined.

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 PreDictor™)	Magnetic beads (Mag Sepharose)	HiTrap™ column	HiScreen™ column
Icon on page 5							
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 ÄKTAxpress 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

If you need further guidance for product selection, download our free Purify App cytiva.com/purify

Ordering information

BACK TO RESIN SELECTION GUIDE

Protein A ligand-based resins

Resin	Main feature	Particle size, d_{50v}* :	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	25 mg/column	1 mL	1 column	29048576
							2 columns	17040203
							5 columns	17040201
					125 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 favor 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	28 mg/column	1 mL	2 columns	17507902
							5 columns	17507901
					140 mg/column	5 mL	1 column	17508001
							5 columns	17508002
			Gravity flow column	rProtein A GraviTrap	28 mg/column	1 mL	10 columns	28985254
MabSelect PrismA	An excellent choice when purifying multiple types of antibodies, because the resin can be thoroughly cleaned between runs to minimize the risk of cross-contamination. The alkali-tolerant protein A ligand allows use of 0.5 to 1.0 M NaOH. MabSelect PrismA has very high dynamic binding capacities at most commonly used residence times.	~ 60 µm	Resin in bulk	MabSelect PrismA	80 mg/mL resin	25 mL	1 bottle	17549801
						200 mL	1 bottle	17549802
			HiTrap column	HiTrap MabSelect PrismA	40 mg/column	1 mL	1 column	17549851
							5 columns	17549852
				HiTrap MabSelect PrismA	200 mg/column	5 mL	1 column	17549853
							5 columns	17549854
			HiScreen column	HiScreen MabSelect PrismA	188 mg/column	4.7 mL	1 column	17549815

Protein A ligand-based resins (continued)

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Resin	Main feature	Particle size, d_{50v}* :	Format	Product name	Approx. binding capacity**	Volume	Pack size	Product code
MabSelect SuRe	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).	~ 85 µm	Resin in bulk	MabSelect SuRe	50 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	30 mg/column	1 mL	5 columns	29268402‡
					150 mg/column	5 mL	5 columns	29157185‡
			HiScreen column	HiScreen MabSelect SuRe LX	141 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	Not tested	1 mL	5 columns	17549111‡
					Not tested	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. Dynamic binding capacity for human IgG. Determined at 6 min residence time for resin in bulk.
† 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

BACK TO RESIN SELECTION GUIDE

Resin	Main feature	Particle size, d_{50v}*:	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

* Median particle size of the cumulative volume distribution
** According to recommended protocols

IgM and IgY purification resins

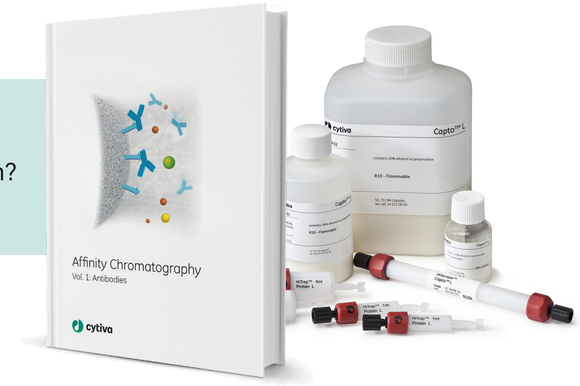
BACK TO RESIN SELECTION GUIDE

Resin	Main feature	Particle size, d_{50v}*:	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	17511101

* 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 cytiva.com/ProteinHandbooks



Antibody fragment purification resins

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Resin	Main feature	Particle size, d_{50v}* :	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 µm	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
					125 mg human Fab/column	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 µL	N/A†	6 µL	4 plates	17547830
				PreDictor Capto L, 20 µL	N/A†	20 µL	4 plates	17547831
				PreDictor Capto L, 50 µL	N/A†	50 µL	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 µm	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 µL	N/A†	6 µL	4 plates	17548213‡
				PreDictor LambdaFabSelect, 20 µL	N/A†	20 µL	4 plates	17548214‡
				PreDictor LambdaFabSelect, 50 µL	N/A†	50 µL	4 plates	17548215‡

* 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).
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Antibody fragment purification resins (continued)

BACK TO RESIN SELECTION GUIDE

Resin	Main feature	Particle size, d_{50v}*:	Format	Product name	Approx. binding capacity**	Volume	Pack size	Product code
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 µm	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 µL	N/A†	6 µL	4 plates	28980195‡
				PreDictor KappaSelect, 20 µL	N/A†	20 µL	4 plates	28980196‡
				PreDictor KappaSelect, 100 µL	N/A†	100 µL	4 plates	28952733‡

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