MILLIPORE

Montage® Antibody Purification Kits

Fast and Convenient PROSEP®-A and PROSEP-G Spin Column Kits for Antibody Purification

- *Available with immobilized Protein A or Protein G*
- *Easy-to-use*
- *Antibody purification in <60 minutes*
- *High yields of purified antibodies*

Total Purification Solution

Montage Antibody Purification Kits are designed for fast and easy antibody purification from serum, ascites or cell culture supernatants. The kits offer advantages at each stage of the antibody purification process – from sample prep to concentration of purified antibodies.

The kits include ready-to-use Montage Spin Columns pre-packed with either PROSEP-A or PROSEP-G media plugs for antibody purification processing by centrifugation.

The kits also include Steriflip® filter devices for prefiltration, Amicon® Ultra devices for desalting and concentrating purified antibodies, and optimized buffer solutions for each stage of the purification process.

Rapid and Convenient

Unlike traditional gravity flow columns for laboratory-scale processing, Montage PROSEP-A and PROSEP-G Spin Columns offer a quick and simple method of antibody purification by centrifugation. The pre-packed media plugs provide time savings and ensure consistent, reproducible results.

Reliable

Montage Spin Columns incorporate PROSEP high capacity media for rapid mass transport and a very high dynamic capacity for IgG. The PROSEP family of media has a long history of success in large scale bioprocessing applications, including PROSEP-A for industrial monoclonal antibody purification. Montage Kits now deliver that same level of performance and reliability for research-scale antibody purification.

DATA SHEET

Complete Kit Yields Quality Results

In addition to optimized, ready-to-use buffers, the Montage Kit provides critical tools to take you from sample prep to purified, concentrated antibodies.

Sample Clarification
Prefiltration with Steriflip filter devices maximizes the life of Montage spin columns.

Antibody Purification
Ready-to-use Montage Spin Columns are pre-packed with PROSEP-A or PROSEP-G media for high yields and purity.

Antibody Concentration and Desalting
High-performance Amicon Ultra devices incorporate low-binding Ultracel™ membrane to deliver high yields of purified and concentrated antibody sample in your desired buffer.

Cost-effective

Montage Kits with PROSEP Spin Columns are an economical solution for antibody purification. Steriflip devices included in the kit for prefiltration remove aggregates that can shorten column life. By using Steriflip for prefiltration, Montage Spin Columns are proven successful for regeneration up to 10x (see Figures 1 and 2) with no loss of binding capacity or specificity. The kits provide enough buffers to support 20 purifications per kit, based on 10x use of each spin column.

Performance

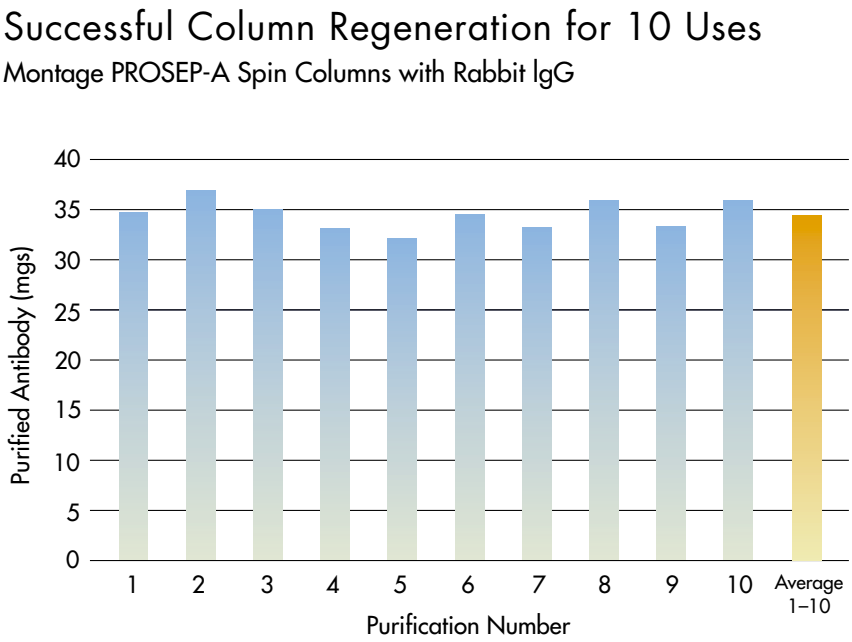

Figure 1. *Rabbit IgG Purifications/ 10X reuse with Rabbit Serum: Rabbit IgG was purified 10 consecutive times from normal rabbit serum using the regenerated PROSEP-A spin column. An average of 34.5 mg of Rabbit IgG was purified over 10 cycles with a CV of 4.1%. The data shows that there is no loss to binding capacity or reduced specificity when columns are regenerated for 10 purifications.*

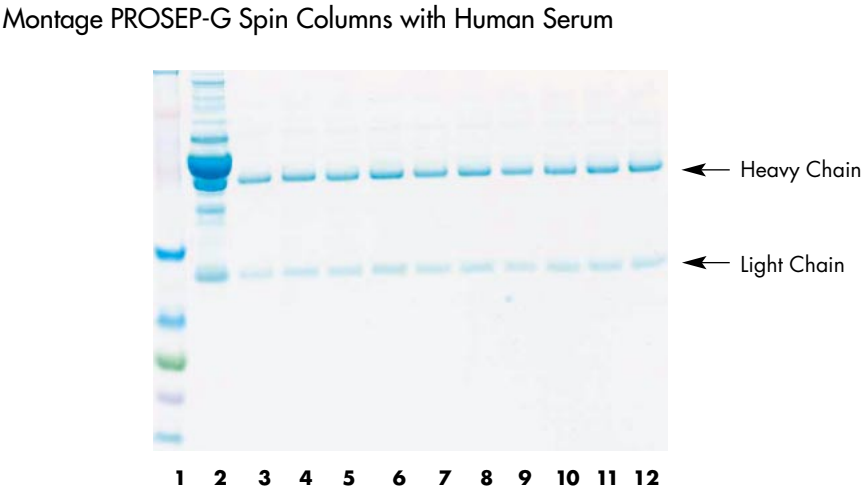

Figure 2. *Human IgG Purifications/10X reuse with Human Serum: Montage PROSEP-G Spin Columns can be regenerated for a total of 10 consecutive times with no loss to binding capacity or reduced specificity. Human IgG was purified 10 consecutive times from normal serum using the regenerated PROSEP-G spin column. An average of 12.96 mg of Human IgG was purified over 10 cycles with a CV of 7.3%.*

Effective Purification of Monocolonal Antibodies

Montage PROSEP-A Spin Columns

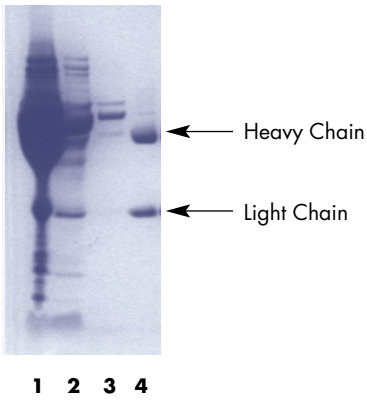

Figure 3. *Mouse MAb Purification from Ascites. Monoclonal mouse IgG was purified from ascitic fluid, courtesy of Exalpha Biologicals, using the Montage PROSEP-A Spin Column with Binding Buffer A and Elution Buffer B2. Lane 1 represents unpurified ascites. Lane 2 represents flow through ascites. Lane 3 represents wash flow through. Lane 4 represent 5 µg purified antibody load. SDS-PAGE analysis validates that the columns can purify monoclonal antibodies from mouse ascites.*

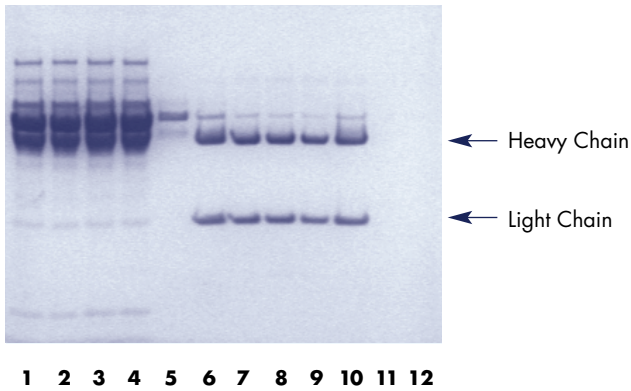

Figure 4. *Mouse MAb Purification from Hybridoma Supernatant. Montage PROSEP-A Spin Columns were used to purify mouse monoclonal antibody from hybridoma cell culture supernatant, courtesy of Exalpha Biologicals, using Binding Buffer A and Elution Buffer B2. Lanes 1–4 represent hybridoma flow through. Lane 5 represents column wash. Lanes 6–10 represent purified antibodies. Lane 11 represents column regeneration, and lane 12 shows column wash. SDS-PAGE analysis confirms that purified mouse IgG can be isolated from cell culture supernatant.*

Effective Purification with Serum from a Variety of Species

Montage PROSEP-G Spin Columns

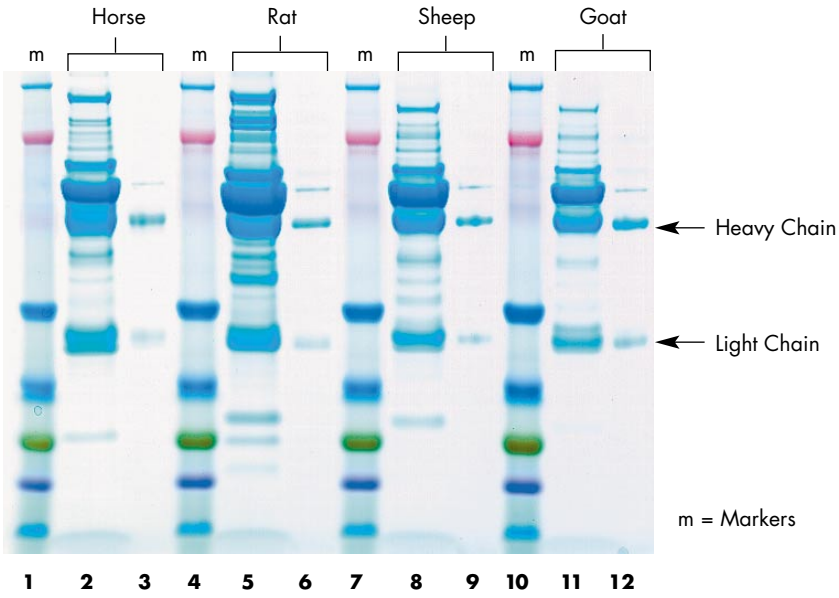

Figure 5. ***Horse, rat, sheep, and goat IgG were purified on PROSEP-G spin column devices using Binding Buffer A and Elution Buffer B2.*** *Lanes 1, 4, 7 and 10 represent markers. Lanes 2, 5, 8 and 11 represent crude serum from horse, rat, sheep and goat respectively. Lanes 3, 6, 9 and 12 represents purified antibodies. SDS Page Analysis confirms that PROSEP-G can purify antibody from a variety of species.*

Purification of Functional Antibodies

Montage PROSEP-G Spin Columns

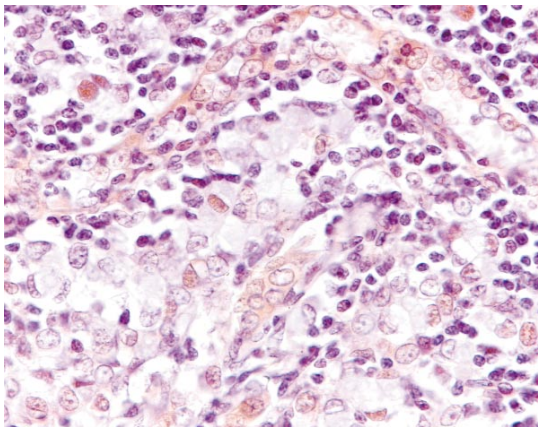

Figure 6. *Human Invasive Breast Carcinoma: Immunohistochemical staining (brown color) for IL-2 in cells lining the lymph ducts using the E 10 B monoclonal antibody (mouse IgG_1 isotype) purified with a Montage PROSEP-G Spin Column. The paraffin section was counterstained with hematoxylin (purple stain). Results courtesy of Dr A. Mobasheri, Faculty of Veterinary Science, University of Liverpool, United Kingdom.*

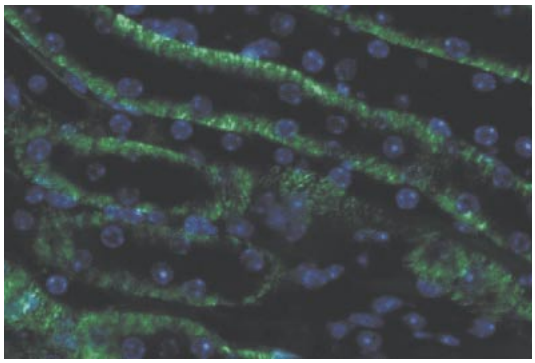

Figure 7. *Mouse kidney: Immunofluorescence localization of the α1 subunit of Na, K-ATPase (green fluorescence) in the mouse renal medulla using the α5 monoclonal antibody (DSHB, University of Iowa) purified with a Montage PROSEP-G Spin Column. The secondary antibody was FITC-conjugated goat anti mouse IgG (Sigma). Nuclei were counterstained (blue fluorescence) using DAPI (Vector Laboratories). Results courtesy of Dr A. Mobasheri, Faculty of Veterinary Science, University of Liverpool, United Kingdom.*

Binding Affinities of Protein A and Protein G

Protein	A	G
Human IgG_1	++	++
Human IgG_2	++	++
Human IgG_3	–	++
Human IgG_4	++	++
Human IgA	+	–
Human IgD	+	–
Human IgE	+	–
Human IgM	+	–
Mouse IgG_1	+	+
Mouse IgG_{2a}	++	++
Mouse IgG_{2b}	++	++
Mouse IgG_3	+	++
Mouse IgM	+/–	–
Rat IgG	++	++
Rat IgG_1	+/–	+
Rat IgG_{2a}	+/–	++
Rat IgG_{2b}	+/–	+
Rat IgG_{2c}	+/–	+
Rat IgM	+/–	–
Rabbit IgG	++	++
Hamster IgG	+	++
Guinea Pig IgG	++	+
Bovine IgG	+	+
Sheep IgG	+/–	+
Goat IgG	+/–	+
Pig IgG	++	++
Chicken IgG	–	+/–
Fragments		
Human Fab	+	+
Human $F(ab')^2$	+	+
Human scFv	+	–
Human Fc	+	+
Human κ	–	–
Human λ	–	–

++ = Strong affinity

+ = Moderate/slight affinity

+/– = Requires evaluation

– = No affinity

Table 1. *Relative affinity of Protein A and Protein G.*

Binding Capacities

Typical Static Binding Capacities on PROSEP-A

Polyclonal Antibodies

Species	*Capacity (mg/mL)**
Bovine	27
Goat	17
Guinea Pig	38
Human	40
Mouse	33
Porcine	38
Rabbit	37
Rat	13
Sheep	13

Monoclonal Antibodies

Subclass	*Capacity (mg/mL)**
Mouse IgG_1 κ	13
Mouse IgG_{2a}	30
Mouse IgG_{2b}	22
Mouse IgG_3	36
Mouse IgG_3 λ	28
Mouse IgG_1	15

** mg antibody/mL packed PROSEP-A media*

Table 2. *Typical static capacities of PROSEP-A media for a range of polyclonal and monoclonal antibodies. Polyclonal antibodies were adsorbed from PBS pH 7.4 and eluted with 0.1M glycine/HCl pH 3.5–3.0. Monoclonal antibodies were adsorbed from 0.1M borate buffer pH 8.5 containing 0.15M NaCl and eluted with 0.1M citrate pH 6.0–3.0. NOTE: Devices provided can only be run in dynamic mode. For example, each device typically purifies 20–40 mg of rabbit IgG in dynamic mode.*

Typical Static Binding Capacities on PROSEP-G

Polyclonal Antibodies

Species	*Capacity (mg/mL)***
Human IgG	20-24

***mg antibody/mL packed PROSEP-G media*

Monoclonal Antibodies

Subclass	*Capacity (mg/mL)***
Mouse IgG_1	8-10
Human IgG_3	5-7
Rat IgG_{2b}	8-10

Table 3. *Typical static capacities of PROSEP-G media.*

Specifications

Source	**Recombinant Protein A expressed in *E. coli***	**Recombinant Protein G expressed in *E. coli***
Supporting Matrix	Porous glass	Porous glass
Media Particle size	75 to 125 microns	74 to 125 microns
Media Bed Volume	1.6 mL	1.6 mL
Binding Capacity	≥ 20 mg Rabbit IgG	≥10 mg Rabbit IgG
Recommended Working pH	2.0 to 9.0	2.0 to 9.0
Maximum volume in swinging bucket rotor	20 mL	20 mL
Storage Conditions	2–8 °C for media. Buffer may be stored at room temp.	2–8 °C for media. Buffer may be stored at room temp.
Color Coded End-caps	Red	Yellow

Ordering information

Montage Antibody Purification Kits with PROSEP-A or PROSEP-G

Kit Contents:

- 2 media plugs in 20% ethanol
- 2 Montage Spin Columns
- 4 Centrifuge tubes/caps
- 1 Insertion tool
- 4 Steriflip devices
- 4 Amicon Ultra devices
- 2 Binding buffer A (500 mL each)
- 1 Elution buffer B1 (250 mL)*
- 1 Elution buffer B2 (500 mL)
- 1 Neutralization buffer C (50 mL)
- 1 Protocol card

** PROSEP-A Kit only*

Montage Kits include components listed above (to support approximately 20 purifications).

Description	Catalogue No.
Montage Antibody Purification Kit with PROSEP-A	LSK2 ABA 20
Montage Antibody Purification Kit with PROSEP-G	LSK2 ABG 20

Also available

Montage Spin Columns with PROSEP-A or PROSEP-G can be purchased separately. Each pack includes 6 media plugs, 6 spin column devices, 12 centrifuge tubes/caps, an insertion tool and a protocol card. Steriflip devices and Amicon Ultra can be purchased separately. For more information, visit millipore.com/montagePROSEP.

Description	Qty/Pk	Catalogue No.
Montage Spin Columns with PROSEP-A	6 spin columns	LSK2 ABA 60
Montage Spin Columns with PROSEP-G	6 spin columns	LSK2 ABG 60
Steriflip-GP .22 µm	25	SCGP 005 25
Amicon Ultra-15 (30K membrane)	8 24 96	UFC9 030 08 UFC9 030 24 UFC9 030 96

To Place an Order or Receive Technical Assistance

For additional information call your nearest Millipore office:
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Lit. No. PF1232EN00 Rev. A
Printed in U.S.A. 1/03 03-023
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