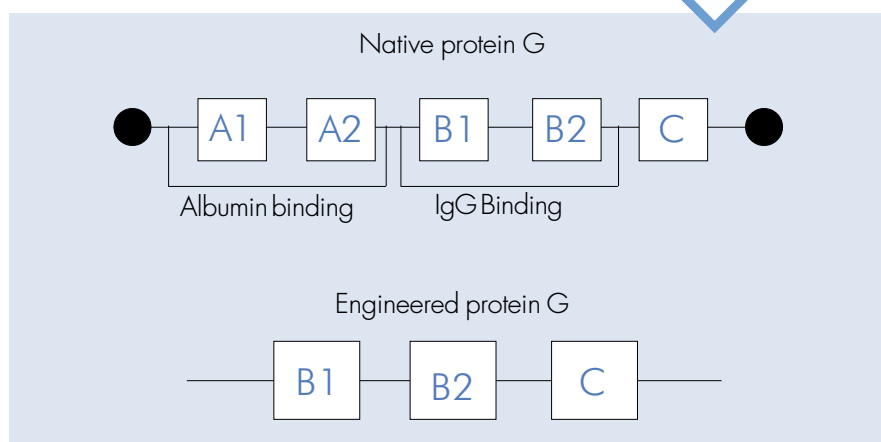


PROSEP[®]-G Chromatography Media

Designed for the purification of antibodies which do not bind efficiently to PROSEP-A High Capacity.

Certificate of Analysis for this product provided in every box.

PROSEP-G is designed for the purification of the small number of antibodies which do not bind efficiently to PROSEP-A High Capacity. Examples of these antibodies include human IgG₃, rat IgG's and very low affinity mouse IgG₁. PROSEP-G can also be applied to the purification of some antibody fragments, and the removal of contaminating antibodies, such as bovine IgG from fetal calf serum, to very low levels. Another application of PROSEP-G lies in the analytical area for the detection of IgG using FPLC or HPLC instruments.



Ligand (Protein G)

Protein G used in the manufacture of PROSEP-G is a recombinant engineered form of Streptococcal protein G. It binds to the constant region of many types of immunoglobulin G. Protein G contains two IgG-binding 'B domains' and the highly charged 'C region'. The region of native protein G responsible for albumin binding has been eliminated.

Specificity of Protein G for Proteins

Human IgG	++
Mouse IgG	++
Rat IgG	+
Rabbit IgG	++
Sheep IgG	++
Horse IgG	++
Bovine IgG	++
Guinea Pig IgG	++
IgA	-
IgM	-
IgGE	-
IgGD	-
Chicken IgY	-
Albumin	-

++ Strong binding
+ Intermediate binding
- No binding

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Matrix

PROSEP consists of glass particles permeated by interconnecting pores of uniform and precisely controlled size. The nature of this matrix allows high flow rates at relatively low back pressures. As there is a linear relationship between back pressure and flow rate, the response of a PROSEP packed column to increased flow rate is entirely predictable over different column lengths.

Capacity

PROSEP-G binds antibodies over a pH range from 4 – 8 and the bound antibodies are eluted over a pH range from 3 – 2. Typical binding capacities for a number of different antibodies are shown here.

Typical Antibody Binding Capacities on PROSEP-G

Human polyclonal IgG	20 – 24 mg/ml
Mouse IgG ₁ MAb	8 – 10 mg/ml
Human IgG ₃	5 – 7 mg/ml
Rat IgG _{2b} MAb	8 – 10 mg/ml

Manufacturing Standards and Quality Assurance

Millipore recognizes the importance of providing regulatory support and meeting industry quality standards. All PROSEP products are manufactured in laboratories certified to internationally recognized standard, BS EN ISO9001 for the manufacture of chromatography media. The facility is subject to routine independent surveillance audits.

Cleaning and Sanitization

Sustained column performance depends on the use of recommended handling and cleaning procedures. A number of sanitants and cleaning agents are recommended for use with PROSEP-G, for example 0.3% (v/v) hydrochloric acid pH 1.5; 1% (v/v) phosphoric acid pH 1.5; 6 M guanidine HCl acid. Cleaning solutions such as 80% ethanol/0.5 M acetic acid can also be used with PROSEP-G in situations where discoloration of the adsorbent might occur, e.g. purification of IgG from plasma. These are detailed in the Operating Instructions which accompany the product.

Storage and Handling

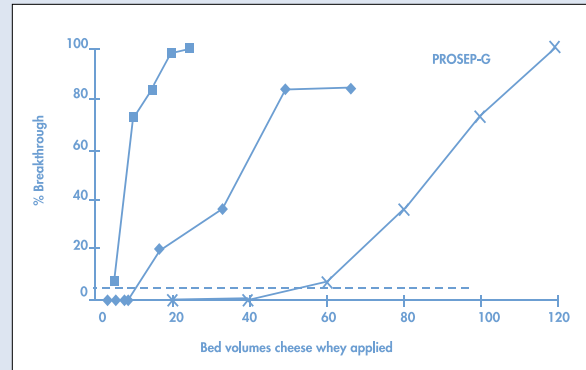
PROSEP-G is supplied in 0.1 M acetate buffer containing 0.5 M NaCl pH 5.2 and a suitable preservative. During use PROSEP-G may be stored in PBS or other suitable buffer containing a preservative. Store between 2 - 8 °C.

Ordering Information

PROSEP-G	Catalogue Number
2 mL prepacked column	115113522
2 mL	115113322
10 mL	115113324
50 ml	115113326
100 ml	115113327
500 ml	115113329
1 litre	115113330
5 litre	115113333
10 litre	115113335

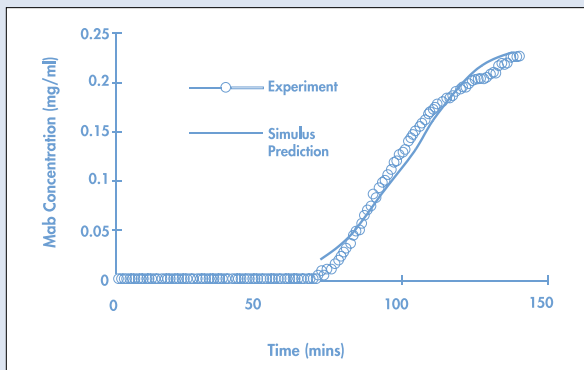
Applications

PROSEP-G has been used in the purification of a variety of antibodies from a range of feedstocks and bovine IgG has been purified directly from cheese whey using PROSEP-G. In this latter application, PROSEP-G showed the highest binding capacity compared to a number of different adsorbents. The binding capacity obtained was 7 mg/ml, and the breakthrough curve for the purification is shown here.

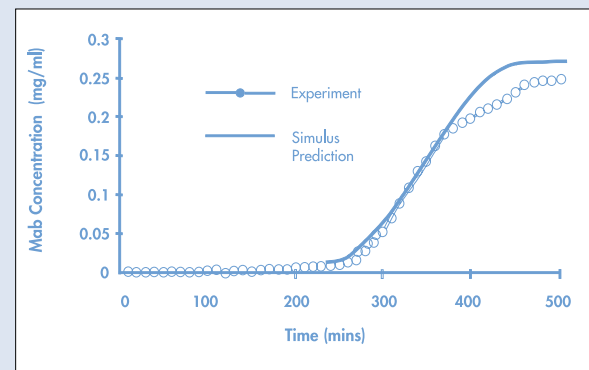


Bovine IgG breakthrough curves for adsorbent A (■), Thiophilic adsorbent (◆) and PROSEP-G (×), at a flow rate of 200cm/hr.

Effective separation and purification of antibodies can be achieved with PROSEP-G both in packed bed and fluidized bed. Purification of a monoclonal antibody using PROSEP-G in packed and fluidized bed has been studied by Wiblin et al, [J. Chromatography, 702 (1995) 81 – 8], and showed similar breakthrough curves for both operations.

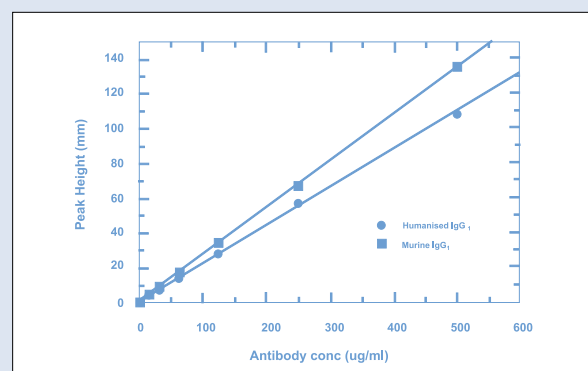


Comparison between the adsorption of IgG onto PROSEP-G using a packed bed and the results fitted by Simulus (a mathematical model for predicting adsorption kinetics in chromatographic separations).



Comparison of adsorption of IgG onto PROSEP-G using an affinity fluidised bed and prediction given by Simulus

PROSEP-G can also be applied to the determination of antibodies in culture media with many advantages over classical immunoassay techniques such as ELISA. Cycle time is very short, permitting analysis in less than 20 minutes. Setup, calibration and validation are simple and quick using standard FPLC or HPLC instruments. The figure here shows a typical standard curve for two different antibodies.



Standard curves obtained by injecting known amounts of antibody to PROSEP-G

Millipore Chromatography

Millipore offers a full range of chromatography products for laboratory, pilot and production scale separations.

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Unpacking System



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