

# MacroCap SP

MacroCap™ SP is a cation exchanger designed for the purification of large biomolecules such as polyethylene glycol (PEG)-modified proteins (i.e., PEGylated proteins) that are intended for use as biopharmaceuticals.

PEGylation typically changes a native protein sample into a mixture of native protein and much larger-sized PEG-protein conjugates of varied PEG-to-protein mole ratios. Because PEG is neutral, such conjugates exhibit lower average surface charge. In post-PEGylation purification steps, these two factors (larger size and lower charge) adversely affect capacity and resolution (1). These factors also contribute to fouling of the medium, which reduces its lifetime. MacroCap SP (Fig 1) overcomes these issues.

## MacroCap SP provides:

- High purity and yield of PEGylated proteins at high sample loads
- Good cleaning-in-place (CIP) stability for long medium lifetime and economical operation

## Medium characteristics

### Designed to separate large biomolecules

MacroCap SP is based on GE Healthcare's proprietary media with mass transfer properties suitable for large biomolecules. The base matrix is highly porous, which gives high available surface area for adsorption of large molecules. Basic characteristics are shown in Table 1.

### High purity and yield at high sample load

MacroCap SP is designed to separate PEGylated and other large biomolecules. It allows separation of mono- from oligo- and non-PEGylated proteins with high selectivity under high load conditions. Figure 2 shows the separation of cytochrome C modified with 20 000 M<sub>r</sub> PEG on MacroCap SP, at a sample load of 6 mg protein/ml medium. The dynamic binding capacity ( $Q_{B10\%}$ ) for mono-PEGylated cytochrome C was 3.8 mg/ml.



**Fig 1.** MacroCap SP cation exchanger can be used to purify PEGylated and other large biomolecules to high purity and yield at high sample loads. Robust chemical stability helps ensure a long medium lifetime.

Since PEGylation generally involves pure native protein, the economic value of the product is typically very high. Thus good recovery of target PEGylated protein is of primary importance for overall productivity. The results show that based on absorbance, 99% of the mono-PEGylated protein could be recovered from MacroCap SP at a purity by size analysis of 93%.

The large pore size also makes MacroCap SP suitable for binding other large proteins. Figure 3 compares binding of IgM (M<sub>r</sub> 750 000) to MacroCap SP and SP Sepharose™ High Performance.



**Table 1.** Key characteristics of MacroCap SP

Matrix	Cross-linked copolymer of allyl dextran and N,N-methylene bisacrylamide
Ion exchange type	Strong cation
Charged group	-SO <sub>3</sub> <sup>-</sup>
Total ionic capacity	0.10 to 0.13 mmol H <sup>+</sup> /ml medium
Particle size <sup>1</sup>	50 μm (d <sub>50V</sub> )
Flow velocity	120 cm/h in BPG 300, 20-cm bed height or 70 cm/h in BPG 300, 30-cm bed height using process buffers with the same viscosity as water at < 3 bar (0.3 MPa)
Recommended separation range	a) Proteins in excess of 150 000 M <sub>r</sub> b) Functionalized dextrans or PEGs greater than or equal to 20 000 M <sub>r</sub> c) PEGylated proteins containing greater than or equal to 10 000 M <sub>r</sub> of PEG (total) per conjugate.
pH stability <sup>2</sup>	
short-term	2 to 13
working	3 to 12
long-term	4 to 11
Storage temperature	4°C to 30°C
Chemical stability	All commonly-used aqueous buffers, 0.5 M NaOH, 0.1 M citric acid, 25% ethanol, 30% propanol, 30% methanol, 50% ethylene glycol, 1% Tween™-20, and 1% SDS.

<sup>1</sup> d<sub>50V</sub> is the medium particle size of the cumulative volume distribution.

<sup>2</sup> Short-term pH: pH interval where the medium can be subjected to cleaning-in-place or sanitization-in-place (accumulated 90 to 400 h at room temperature) without significant change in function.

Working pH: pH interval where the medium binds protein as intended or that needed for elution without adverse long-term effect.

Long-term pH: pH interval where the medium can be operated without significant change in function.

## Good chemical stability and long medium lifetime

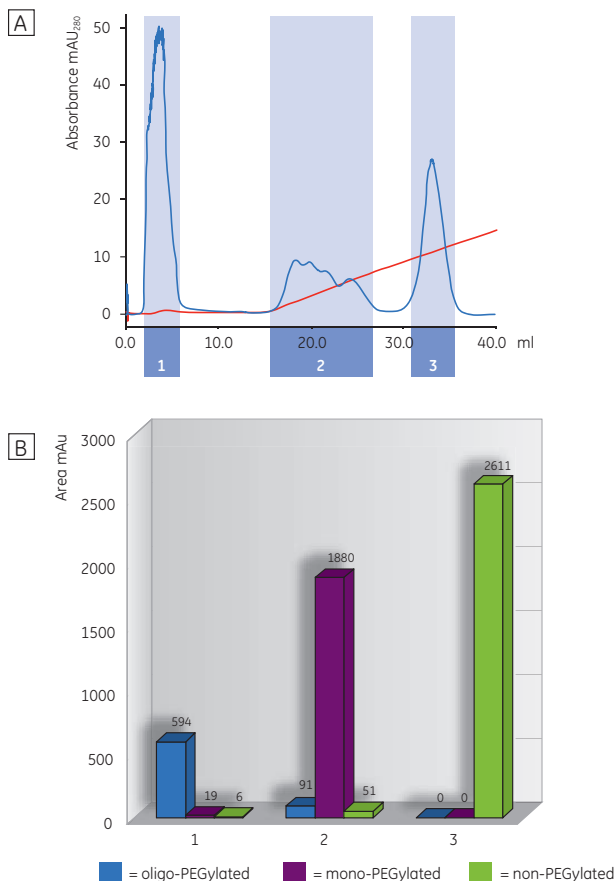
MacroCap SP has good chemical stability, which allows CIP to be performed both at acidic and alkaline conditions. The hydrophilic nature of the MacroCap SP base matrix reduces nonspecific binding and reduces fouling issues that may be experienced with more hydrophobic base matrices. Together these features assure long medium lifetime.

Figure 4 shows that the selectivity and binding capacity of the medium for RNase A, cytochrome C, and lysozyme were unchanged after 30 cycles of CIP involving acidic and alkaline conditions in each cycle.

## Meets industrial needs

MacroCap SP is a GE Healthcare BioProcess™ medium, specifically designed to meet the demands of industrial biotechnology. This means that the medium is scalable from laboratory to production, is produced with validated manufacturing procedures, and can withstand standard CIP and sanitization-in-place procedures. In addition, BioProcess media are supported with regulatory support files and comprehensive documentation, as well as security of supply service.

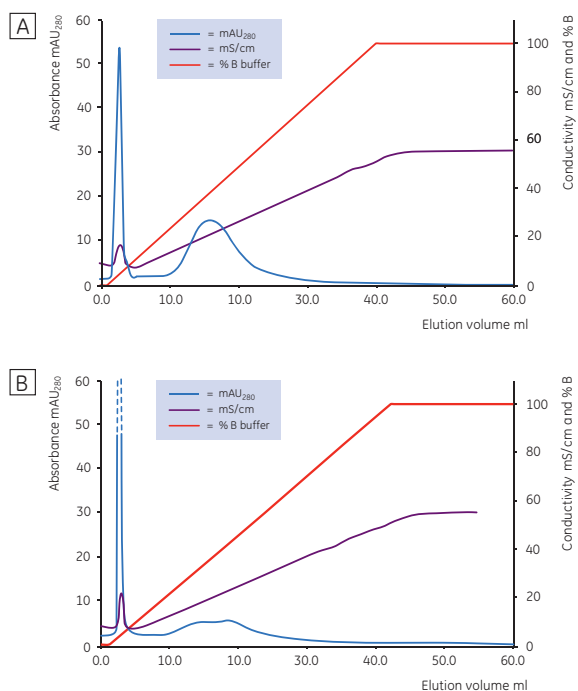
Column: Tricorn 5/100 (bed height 107 mm; column volume (CV) 2.1 ml) packed with MacroCap SP  
Sample: Cytochrome C modified with 20 000 M<sub>r</sub> PEG  
Sample load: 6 mg total protein per ml medium  
Buffer 1: 0.02 M sodium phosphate, pH 6.8  
Buffer 2: Buffer A + 0.4 M sodium chloride  
Flow rate: 0.2 ml/min (61 cm/h)  
Gradient: 0 to 100% Buffer B in 20 CV  
System: ÄKTAexplorer™ 10



**Fig 2.** PEGylated cytochrome C<sup>1</sup> separated on MacroCap SP. **(A)** Chromatograms of the separation. Fractions that were pooled for the size exclusion chromatography (SEC) analysis are indicated in blue and numbered. **(B)** Pooled fractions indicated in **(A)** were analyzed by SEC on Superdex™ 200 for the amounts of oligo-, mono-, and non-PEGylated proteins.

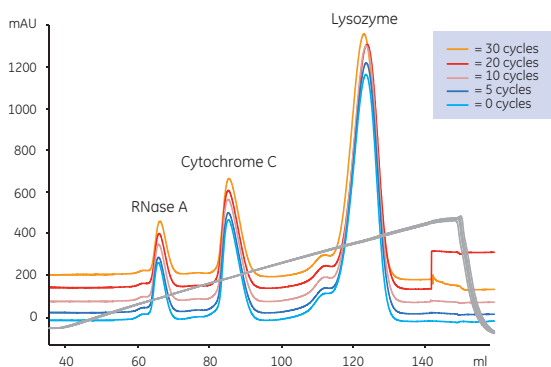
<sup>1</sup> Bovine cytochrome C (Sigma Aldrich, USA) covalently modified with monomethoxy-PEG 20 000 succinimidylpropionic acid (SPA) reagent (Nektar Therapeutics, USA).

Column: Tricorn 5/100 packed with either (A) MacroCap SP or (B) SP Sepharose High Performance (CV 2 ml)  
 Sample: IgM (human), 96% pure by HPLC  
 Sample load: 0.5 mg/ml medium  
 Buffer A: 100 mM sodium acetate, pH 4.75  
 Buffer B: Buffer A + 0.5 M sodium chloride  
 Flow rate: 0.3 ml/min (90 cm/h)  
 Gradient: 0 to 100% Buffer B in 20 CV  
 System: ÄKTApexplorer 10



**Fig 3.** Binding and elution of a pure (96% by HPLC) sample of IgM ( $M_n = 750\ 000$ ) (Sigma Aldrich, USA) on **(A)** MacroCap SP and **(B)** SP Sepharose High Performance. The large pore structure of MacroCap SP gives it a greater capability to bind large biomolecules.

Column: MacroCap SP in 10 mm i.d., 9 cm bed height column (CV 7.1 ml)  
 Sample: 0.5 mg/ml RNase A, 0.5 mg/ml cytochrome C and 0.5 mg/ml lysozyme  
 Sample load: 1 CV  
 Buffer A: 20 mM phosphate buffer at pH 6.8  
 Buffer B: Buffer A + 0.4 M NaCl  
 Flow rate: 1 ml/min (75 cm/h)  
 Gradient: 0% to 100% Buffer B in 15 CV  
 System: ÄKTApexplorer 100



**Fig 4.** CIP study of MacroCap SP. The performance of MacroCap SP in the separation of RNase A, cytochrome C, and lysozyme was unaffected following 30 cycles of a CIP procedure. CIP conditions per cycle: 5 column volumes (CV)  $H_2O$ , 2 CV 0.5 M NaOH followed by 40 min static contact, 2 CV  $H_2O$ , 2 CV acidic solution ( $\sim$ pH 2) followed by 40 min static contact, 2 CV  $H_2O$ , 5 CV 0.5 M NaOH followed by 40 min static contact, and 5 CV  $H_2O$ .

## Operation and method development for PEGylated proteins

### How PEGylation affects protein purification

PEGylation changes a native protein into a much larger-sized PEG-protein conjugate of lower average surface charge. By weight, PEG polymers typically occupy over six times the hydration volume of globular proteins. Both effects (larger size and lower surface charge) increase with the degree of PEGylation. Consequently, the dynamic binding capacity (mg of protein per ml gel) of an ion exchange medium for PEGylated proteins, compared with native proteins, can decrease by a factor of about 10. In a typical ion exchange gradient run with a PEGylation reaction mixture, the products will elute in the following order: free PEG substances, oligo-PEGylated proteins, mono-PEGylated proteins, and non-PEGylated proteins.

During sample loading, proteins with higher surface charge will often displace those with lower charge, and non-PEGylated proteins exhibit some capacity to displace PEGylated proteins at higher sample loading. Ease of displacement is related to the degree of PEGylation and other factors such as solution conductivity. As a result, greater loading may contribute to higher target purity in such situations.

The above factors suggest that loading PEGylation reaction mixtures using buffers with a conductivity that promotes flow through of non-target PEGylated proteins, and optimal binding of target and non-PEGylated proteins, will make better use of column capacity.

### Development and optimization

The aim of designing and optimizing a method for the separation of large biomolecules, such as PEGylated proteins, is to ensure high and consistent binding capacity and selectivity, enabling robust and scalable operation.

MacroCap SP operates as a normal cation exchanger. It has been designed for large-scale operation, and allows flow velocities of 120 cm/h at 20-cm bed height in a 30 cm i.d. column (70 cm/h at 30-cm bed height). These are common operating flow velocities for processing of large, slowly diffusing biomolecules. A faster flow may be possible with lower bed heights. Note, however, that residence times of 6 to 15 min are recommended to fully exploit the properties of the medium, mainly because larger molecules diffuse slower and require more time to bind to the medium.

Method development can be done in small columns, such as Tricorn™ columns. A detailed purification protocol is given in the instruction manual. In addition to reducing protein charge density, PEGylation may alter protein pI by 1 unit or more. As a result it is suggested to experiment with adsorption at lower than normal pH and conductivity.

The difference in performance between various ion exchange media for large biomolecules may not be evident at low sample loadings. However, at high sample loadings, the greater capacity and resolution properties of MacroCap SP become evident, as do reduced fouling and other properties that make MacroCap SP the medium of choice to purify large biomolecules, including PEGylated proteins.

## Scale-up

Laboratory methods can be scaled up using small-diameter columns and working up to the final intended large-scale column height and linear flow rate (residence time). Scale-up can be continued by increasing column diameter. Table 1 lists flow rates and other recommendations to consider when scaling up the use of MacroCap SP. Reducing bed height will often allow for the use of a significantly greater flow rate.

## Cleaning-in-place

Stability studies have demonstrated that MacroCap SP resists harsh CIP conditions at both low and high pH. Note that specific CIP protocols should be developed according to the feedstock applied and other related operating conditions.

## Summary

MacroCap SP is a cation exchanger designed to purify PEGylated and other large biomolecules, at high sample load. Mono-PEGylated proteins can be separated to high purity from oligo-PEGylated and non-PEGylated proteins in a single run. Good CIP stability allows long medium lifetime and eliminates fouling issues that may be experienced with more hydrophobic base matrices.

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

GE Healthcare Bio-Sciences AB  
Björkgatan 30  
751 84 Uppsala  
Sweden

## Reference

1. Fee, C. J. and Van Alstine, J. M. PEG-proteins: Reaction engineering and separation issues. *Chem. Eng. Sci.* **61**, 924–939 (2006).

## Ordering information

Product	Pack size <sup>1</sup>	Code number
MacroCap SP	25 ml	17-5440-10
MacroCap SP	100 ml	17-5440-01
MacroCap SP	1 l	17-5440-02
MacroCap SP	5 l	17-5440-03

<sup>1</sup> Larger quantities are available. Please contact GE Healthcare for more information.

## Literature

Literature	Code no.
Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	18-0004-21

ÄKTAexplorer, BioProcess, Drop design, MacroCap, Sepharose, Superdex, and Tricorn are trademarks of GE Healthcare companies. GE, imagination at work, and GE monogram are trademarks of General Electric Company.

Tween is a trademark of ICI Americas Inc.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare that supplies them. GE Healthcare reserves the right, subject to any regulatory and contractual approval, if required, to make changes in specifications and features shown herein, or discontinue the product described at any time without notice or obligation.

© 2006 General Electric Company – All rights reserved.

GE Healthcare Bio-Sciences AB, a General Electric Company.

GE Healthcare Bio-Sciences AB  
Björkgatan 30, 751 84 Uppsala  
Sweden

GE Healthcare Europe GmbH  
Munzinger Strasse 5, D-79111 Freiburg  
Germany

GE Healthcare UK Ltd, Amersham Place  
Little Chalfont, Buckinghamshire HP7 9NA  
UK

GE Healthcare Bio-Sciences Corp.  
800 Centennial Avenue, P.O. Box 1327  
Piscataway, NJ 08855-1327  
USA

GE Healthcare Bio-Sciences KK  
Sanken Bldg. 3-25-1, Hyakunincho  
Shinjuku-ku, Tokyo 169-0073  
Japan

Asia Pacific Tel: +85 65 6 275 1830 Fax: +852 2811 5251 • Australasia Tel: +61 2 9899 0999 Fax: +61 2 9899 7511 • Austria Tel: 01/57606-1619 Fax: 01/57606-1627 • Belgium Tel: 0800 73 888 Fax: 03 272 1637 • Canada Tel: 800 463 5800 Fax: 800 567 1008 • Central, East, & South East Europe Tel: +43 1 982 3826 Fax: +43 1 985 8327 • Denmark Tel: 45 16 2400 Fax: 45 16 2424 • Finland & Baltics Tel: +358 (0)9 512 39 40 Fax: +358 (0)9 512 39 439 • France Tel: 01 69 35 67 00 Fax: 01 69 41 96 77 • Germany Tel: 0761/4903-490 Fax: 0761/4903-405 • Italy Tel: 02 27322 1 Fax: 02 27302 212 • Japan Tel: +81 3 5331 9336 Fax: +81 3 5331 9370 • Latin America Tel: +55 11 5933 7300 Fax: +55 11 3933 7304 • Middle East & Africa Tel: +30 210 9600 687 Fax: +30 210 9600 693 • Netherlands Tel: 0165 580 410 Fax: 0165 580 401 • Norway Tel: 815 65 555 Fax: 815 65 666 • Portugal Tel: 21 417 7035 Fax: 21 417 3184 • Russia & other C.I.S. & N.I.S Tel: +7 (095) 232 0250 Fax: +7 (095) 230 6377 • South East Asia Tel: 60 3 8024 2080 Fax: 60 3 8024 2090 • Spain Tel: 93 594 49 50 Fax: 93 594 49 55 • Sweden Tel: 018 612 1900 Fax: 018 612 1910 • Switzerland Tel: 0848 8028 12 Fax: 0848 8028 13 • UK Tel: 0800 616928 Fax: 0800 616927 • USA Tel: 800 526 3593 Fax: 877 295 8102



imagination at work