

AFFINITY PURIFICATION

Profinia™ Protein A and G Methods

- Preprogrammed methods for affinity purification
- Integrated desalting to neutralize antibodies immediately after elution
- High reproducibility
- Large sample volumes to accommodate even dilute monoclonal antibody samples
- Compatibility with 1 ml or 5 ml Protein A or G cartridges for a wide range of sample volumes
- Large interface screen to guide purification process with minimal training

Purification of Antibodies Using Preprogrammed Methods on the Profinia Protein Purification System

Protein A and G chromatography media are commonly used in antibody purification due to the high binding affinity and specificity of Protein A or G with the Fc region of the antibody. The Profinia protein purification system now features preprogrammed Profinia Protein A and G methods for the affinity purification of monoclonal and polyclonal antibodies, typically immunoglobulins (IgGs), from serum, ascities, or hybridoma cell culture supernatant (Table 1, Figures 1 and 2). Two types of Profinia Protein A and G methods are

available — the Profinia Protein A and G method and the Profinia Protein A and G plus desalting method. Both methods are designed to use either prepacked Protein A or Protein G cartridges. Bio-Scale™ Mini Bio-Gel® P-6 desalting cartridges are used for desalting. The automated system provides an integrated, tandem desalting step immediately following affinity purification, which elutes and stabilizes antibodies in a neutral pH buffer, and generates ready-to-use antibodies. The whole method is easy, fast, and reproducible.

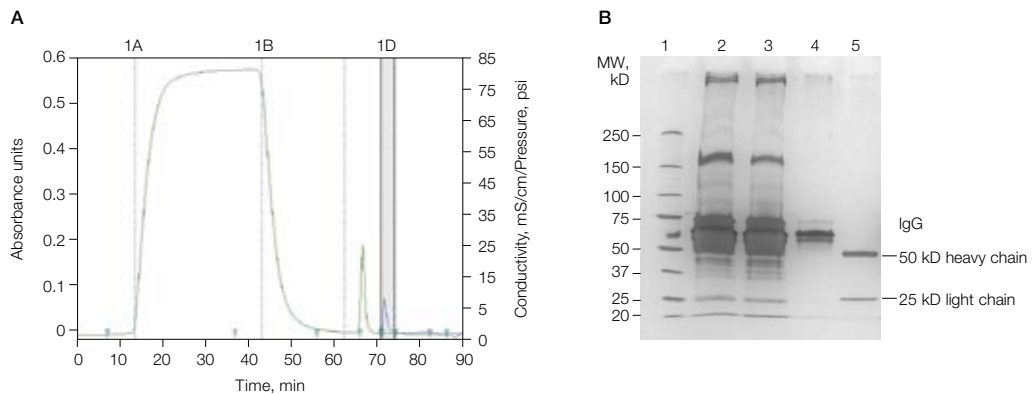


Fig. 1. Purification of 20 ml mouse monoclonal hybridoma supernatant with the preprogrammed Profinia Protein A and G plus desalting method using Bio-Scale™ Mini Affi-Prep® Protein A and Bio-Gel P-6 desalting cartridges. **A**, chromatogram showing different fractions. 1A, flowthrough; 1B, wash; 1D, affinity-purified plus desalted IgG; **B**, silver-stained gel from SDS-PAGE analysis showing Protein A column fractions from the purification of CCRC-M96. Lane 1, Precision Plus Protein™ standards; lane 2, supernatant from hybridoma cell culture; lane 3, flowthrough (1A); lane 4, wash (1B); lane 5, elution (1D). Monoclonal antibody purification data kindly provided by Dr. Michael Hahn, The University of Georgia.



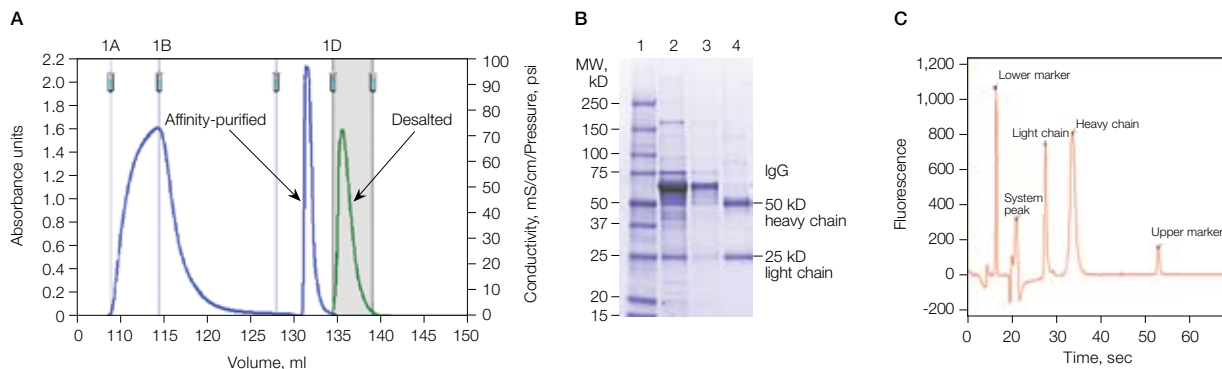


Fig. 2. Purification of mouse IgG with the preprogrammed Profinia Protein A and G plus desalting method using a 1 ml HiTrap HP Protein G cartridge and a 10 ml Bio-Scale Mini P-6 desalting cartridge. **A**, chromatogram showing different fractions. 1A, flowthrough; 1B, wash; 1D, affinity-purified plus desalted IgG; **B**, SDS-PAGE of chromatography fractions. Lane 1, Precision Plus Protein standards; lane 2, flowthrough (1A); lane 3, wash (1B); lane 4, elution (1D); **C**, Experion™ automated electrophoresis system electropherogram analysis of the elution fraction (1D).

Table 1. Antibody binding affinity to Protein A and Protein G.*

Species	IgG Class	Protein A	Protein G
Chicken egg	IgY	—	—
Cow	IgG	—	+
Dog	IgG	+	+
	IgM	+	—
Goat	IgG	+	+++
	IgM	—	—
Horse	IgG	+++	+++
Rabbit	IgG	+++	+++
	IgM	—	—
Rat	IgG	+	++
	IgM	—	—
Sheep	IgG	+++	+++
	IgM	—	—
Mouse	IgG ₁	+	++
	IgG _{2a}	++	++
	IgG _{2b}	++	++
	IgG ₃	+	++
	IgM	++	+
	IgA	++	++
Human	IgG ₁	+++	+++
	IgG ₂	+++	+++
	IgG ₃	—	+++
	IgG ₄	+++	+++
	IgA	+	—
	IgM	+	—
	IgE	+	—

—, No binding; +, weak binding; ++, moderate binding; +++, strong binding.

* Data obtained from *Handbook of Affinity Chromatography* by David S. Hage (ISBN 0824740572). Chapter 14 "Affinity Chromatography in Antibody and Antigen Purification" by Terry M. Phillips.

See bulletin 5701 for recommended buffers for Protein A and G purifications on the Profinia system.

For more information, please visit us on the Web at

www.bio-rad.com/profinia/

Ordering Information

Catalog # Description

Prepacked Bio-Scale Mini Cartridges

732-4600 **Bio-Scale Mini Affi-Prep Protein A Cartridges**, 5 x 1 ml
 732-4602 **Bio-Scale Mini Affi-Prep Protein A Cartridge**, 1 x 5 ml
 732-5304 **Bio-Scale Mini Bio-Gel P-6 Desalting Cartridges**, 5 x 10 ml
 732-5314 **Bio-Scale Mini Bio-Gel P-6 Desalting Cartridges**, 5 x 50 ml
 732-5312 **Bio-Scale Mini Bio-Gel P-6 Desalting Cartridge**, 1 x 50 ml

Profinia Instruments

620-1005 **Profinia Instrument With Accessory Kit and Native IMAC Starter Kit**, 100–240 V, includes cleaning tray, inline filter pack, 2 x 50 ml sample lids, 2 x 15 ml sample lids, bottle starter pack, waste/diluent bottle set, Profinia native IMAC buffer kit, 1 x 1 ml IMAC and 1 x 10 ml desalting cartridge, *E. coli* lysate
 620-1006 **Profinia Instrument With Accessory Kit and GST Starter Kit**, 100–240 V, includes cleaning tray, inline filter pack, 2 x 50 ml sample lids, 2 x 15 ml sample lids, bottle starter pack, waste/diluent bottle set, Profinia GST buffer kit, 1 x 1 ml GST and 1 x 10 ml desalting cartridge, *E. coli* lysate, glutathione reagent

Profinia Systems

620-1010 **Profinia Protein Purification System With Native IMAC Starter Kit**, 100–240 V, includes same as 620-1005 with Profinia software
 620-1011 **Profinia Protein Purification System With GST Starter Kit**, 100–240 V, includes same as 620-1006 with Profinia software

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