Application of UNOsphere SUPrA™ Media, a New Protein A Affinity Chromatographic Support, for Both Laboratory-Scale and Industrial Antibody Purification

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Introduction

Protein A affinity chromatography is a widely used approach for immunoglobulin purification. The relative low cost and high stability of the Protein A media compared to other options for IgG purification make them the most popular choice for antibody purification both at laboratory and industrial scales (Hober et al. 2007).

Bio-Rad's UNOsphere SUPrA affinity media is a recombinant Protein A resin developed for a wide range of research, development, and manufacturing applications where high binding capacity is required. UNOsphere SUPrA affinity media is built on the proven UNOsphere macroporous polymeric matrix. The media has a binding capacity of 25 to 30 mg/ml for IgG in batch mode or column chromatography at process-level flow rates (bulletin 5729). Importantly, UNOsphere SUPrA media has low nonspecific binding and very fast mass transfer properties, making it a flexible and effective tool for any affinity-based capture application.

Here we demonstrate the use of UNOsphere SUPrA media in polyclonal and monoclonal antibody purification in both batch and column chromatography modes and at process-scale flow rates.

Materials and Methods

Chromatography Media

Bio-Scale™ Mini UNOsphere™ SUPrA and Bio-Scale™ Mini Affi-Prep® 1 ml cartridges are from Bio-Rad Laboratories, Inc. nProtein A Sepharose 4 Fast Flow, HiTrap Protein A HP (1 ml cartridge), and MabSelect media are from GE Healthcare group companies.

Immunoprecipitation

Protein A beads (10 µl) were washed twice with 1 ml buffer (20 mM HEPES, pH 7.5, 50 mM KCl, 50 mM NaF, 1 mM active sodium vanadate, 1 mM sodium molybdate, 2 mM imidazole, 250 mM sucrose, and 1 mM EDTA) before the addition of 10 µg of anti-GFP antibody. After incubation at 4°C for 2 hr with constant agitation, 0.5 g of plant tissue extract prepared in 1 ml buffer containing 0.5% Triton X-100 and protease inhibitors (1 mM PMSF, 1x cocktail) were added. After incubation at 4°C for 30 min, the beads were washed four times with 0.5 ml buffer containing 0.1% Triton. Bound proteins were eluted in 50 µl of prewarmed (95°C) SDS sample buffer. A 25 µl aliquot of each eluate, along with the original plant tissue extract, was analyzed by SDS-PAGE and western blot against anti-GFP.

Lab-Scale Antibody Purification

Human serum (HMSRM, Bioreclamation Inc., total protein content 68 mg/ml) was diluted 1:5 in phosphate buffered saline (PBS) and filtered (0.2 micron). IgG was purified from the serum on the Profinia™ protein purification system (Bio-Rad) using the preprogrammed Protein A & G method at a flow rate of 1 ml/min (233 cm/hr, bulletin 5701 and Berardini et al. 2008). Protein A cartridges were equilibrated with 10 column volumes (CV) of PBS, the sample was loaded (5–20 ml) and washed with 12 CV of PBS. IgG was eluted in 4 CV of 0.1 M sodium citrate, pH 3.0.

Protein Analysis

IgG yield was measured for each chromatography run by measuring the absorbance at 280 nm (A_{280} = 1.4 for 1 mg/ml). The purity of the IgG fraction was measured from a Coomassie-stained Criterion™ 4–20% Tris-HCl gel, scanned using a GS-800™ USB-calibrated densitometer, and analyzed by the Quantity One® 1-D software (Bio-Rad).

Monoclonal Antibody Purification

Cell culture supernatant containing monoclonal antibody was processed through SUPrA and MabSelect (GE Healthcare) media packed in 1 ml Bio-Scale Mini™ cartridges using a BioLogic DuoFlow™ chromatography system. After 10 CV of equilibration (1x PBS) and sample loading, the column was washed with 15 CV of 1x PBS. Monoclonal antibody was eluted with 10 CV of 0.1 M glycine, pH 3.0. The flow rate used for all steps was 1.23 ml/min (300 cm/hr).

Results and Discussion

Protein A Mediated Immunoprecipitation of GFP

Anti-GFP antibodies were captured in solution on UNOsphere SUPrA and nProtein A Sepharose 4 Fast Flow media and used to immunoprecipitate GFP from a plant protein extract. Immunoprecipitated GFP was eluted and analyzed by western blotting. Our results indicate that UNOsphere SUPrA and nProtein A Sepharose 4 Fast Flow media both effectively captured the GFP antibody as shown by the strong signal for GFP on the western blot (Figure 1B).

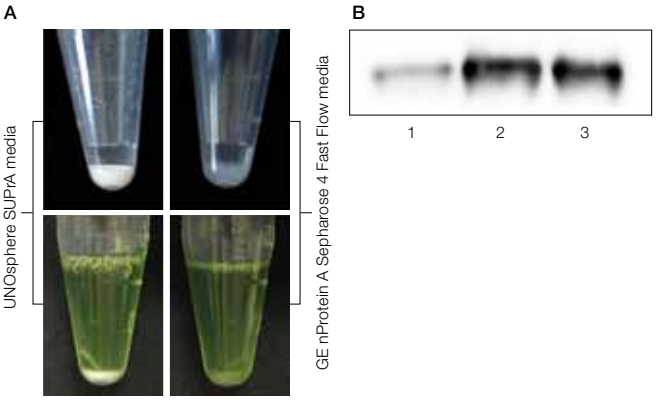

Fig. 1. Capture of GFP by Protein A-GFP antibody. A, Pellet of UNOsphere SUPrA and nProtein A Sepharose 4 fast flow media following centrifugation in plain buffer or crude plant extract. **B**, Western blot analysis of eluate. The presence of GFP in plant extract eluate was detected using anti-GFP polyclonal antibodies. **1**, plant extract; **2**, nProtein A Sepharose 4 Fast Flow eluate; **3**, UNOsphere SUPrA media eluate.

However, if we compare the two different Protein A media when mixed either in plain buffer or with plant extracts, the UNOsphere SUPrA media forms a tight, clearly visible pellet, making washes easier and minimizing the loss of Protein A media during the washes (Figure 1A).

UNOsphere SUPrA Media Binding Capacity for IgG

Different volumes of diluted human serum (5, 10, 15, and 20 ml) were loaded onto 1 ml UNOsphere SUPrA, HiTrap Protein A HP, and Affi-Prep Protein A cartridges (Figure 2A). Purified IgG fractions were analyzed on SDS-PAGE for quantitation and purity assessment (Figure 2). The three different media tested effectively captured the IgG antibody from human serum at 89% purity for both the SUPrA and HiTrap Protein A HP cartridges and at 88% purity for the Affi-Prep Protein A cartridge (Figures 2A and B). However, UNOsphere SUPrA media demonstrated the highest yield at each given sample load.

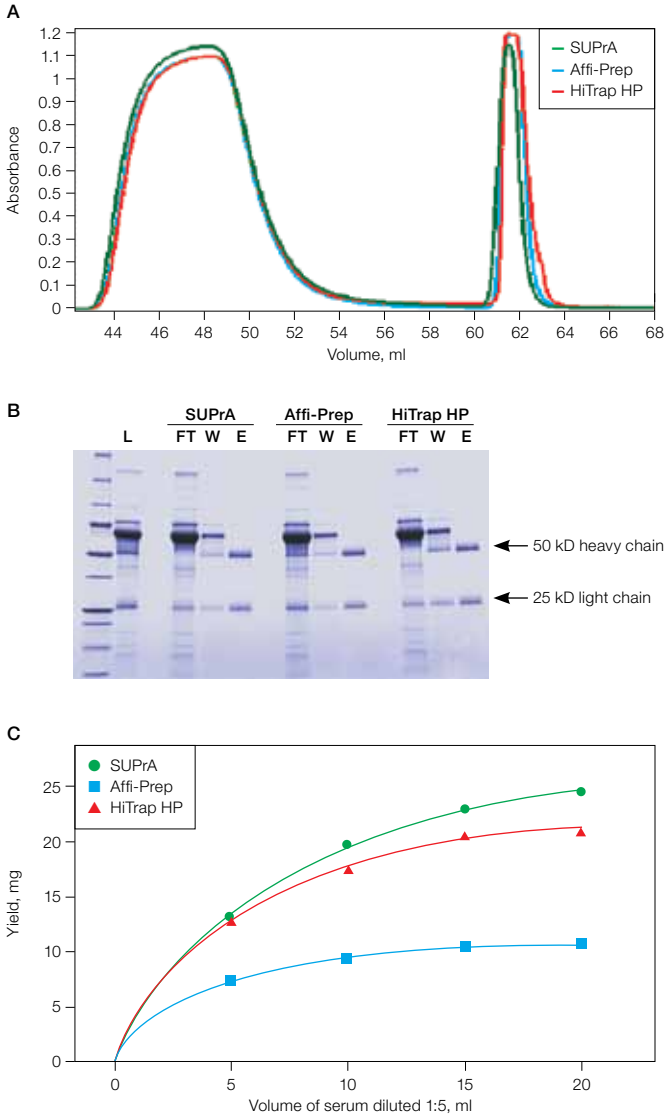

Fig. 2. Lab-scale purification of human IgG from serum using the Profinia protein purification system. A, Chromatogram showing overlayed purification profiles of IgG using three different Protein A media. **B**, SDS-PAGE of purified IgG using SuPrA, Affi-Prep, and HiTrap HP cartridges. L, load; FT, flowthrough; W, wash; E, eluate. **C**, Plots of dynamic yield of IgG purified using different Protein A media cartridges. Data points represent the average of duplicate purifications.

Purification of Monoclonal Antibody with UNOsphere SUPrA Media

UNOsphere SUPrA media is also very effective for the purification of monoclonal antibodies. We compared the performance of UNOsphere SUPrA media with that of MabSelect Protein A media. The results indicate that both media yield similar amounts and purity of mAb, and contain only low levels of host cell proteins and DNA contamination (Figure 3 and Table 1). In a previous technote (He et al. 2008) we demonstrated that UNOsphere SUPrA can serve as an effective mAb capture media for process scale applications. When combined with polishing steps using UNOsphere Q and CHT (ceramic hydroxyapatite) media this three-step workflow produces mAbs of high quality and purity with very little contamination by antibody dimers or aggregates (He et al. 2008).

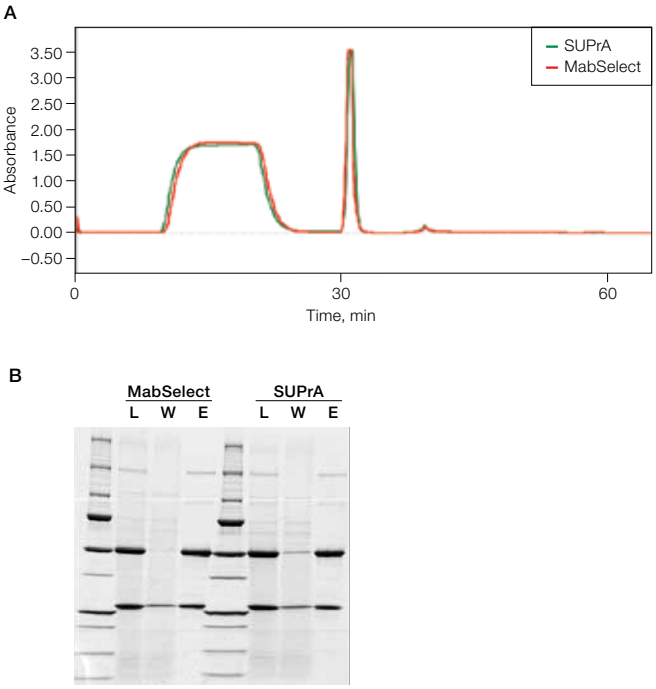

Fig. 3. Purification of monoclonal antibody with MabSelect and UNOsphere SUPrA Protein A media. A, Chromatogram showing overlayed purification profiles of mAb using SUPrA and MabSelect media. **B,** SDS-PAGE of chromatography fractions. L, load; W, wash; E, elution.

Table 1. Quantitation of remaining HCP* and DNA from a single-step monoclonal antibody purification by UNOsphere SUPrA and MabSelect media.

Media	HCP, ng/mg	DNA, ng/mg	rProtein A, ppm
MabSelect	39.2	26.0	6.4
SUPrA	33.2	21.6	20.5

* HCP, host cell protein.

In summary, UNOsphere SUPrA media provides similar dynamic binding capacity and purity to leading competitor products over a range of different laboratory applications. It is an excellent choice for use in protein immunoprecipitation assays and for both laboratory and process-scale purification of polyclonal and monoclonal IgG antibodies.

References

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