Separation of Mouse IgG subclasses by Affinity chromatography on protein-A sepharose

Not all murine and rat Ig classes and subclasses bind to protein-A. In the mouse the binding avidity is in the following order: IgG2b > IgGa = IgG3 >> IgG1 while IgM and IgE fail to bind. In the rat the only IgG subclasses that bind are IgG 2c and to a lower degree IgG1.
In the mouse the binding is Ph dependent (IgG1 bind at pH 8.0 and not at pH 7.2).
The difference in binding affinity of the various Ig's classes to protein-A at different pH is being utilized for stepwise purification of the different classes.
The following protocol is adopted from the Basel Institute Manual. Note that such procedure can be used for the purification of mAb from culture supernatant if the hybridomas were grown in the presence of FCS that do contain Ig or in a gammaglobulin-free horse serum.

Reagents

A. Protein A-Sepharose CL-4B (SPA-Sepharose, Pharmacia, Sweden)

B. Phosphate buffers : 0.5M pH 8.0
0.1M pH 8.0

C. Citrate buffers: 0.1M pH 6
0.1M pH 4.5
0.1M pH 3

D. 0.1M acetic acid in 0.15M NaCl.

E. PBS: 0.01M phosphate, 0.15M NaCl.

Procedure

Swell SAP-Sepharose (pharmacia, Sweden) in 0.1M phosphate pH 8.0 for 30 min, then pack in a clumn in a syringe. Abed volume of about 5ml gel (1.5g dry SpA-Sepharose) has a total capacity of about 20mg of mouse immunoglobulins, but a lower capacity (5-6mg) for IgG1.
Add to 4 volumes of the serum, one volume of 0.5M pH 8 phosphate buffer.

Apply the serum, and let it absorb slowly into the column. Apply phosphate buffer and wash the column until no proteins are detectable in the effluent. This requires a volume of buffer which is about 5 times the volume of the applied serum.
For elution of total IgG, apply 0.1M acetic-acid (sol.D) (about three times the volume of applied serum). For differential elution of IgG subclasses, apply:

Citrate buffer pH 6.0 = elution of the bulk of IgG1
Citrate buffer pH 4.5 = elution of the bulk of IgG2a and IgG3, and some IgG1
Citrate buffer pH 3 = elution of the bulk of IgG2b and some IgG2a.

The eluted proteins are collected into tubes containing 1M K2HPO (1 volume per 5 volumes eluate) and dialyzed against PBS.
After elution, the adsorbent is washed with 0.1M phosphate buffer, pH 0.8, and can be re-used many times. The procedure is performed at room temperature. When not in use, the adsorbent is kept at 4ºC, and sodium azide (10mM) is added to the buffer to prevent bcterial and sodium azide (10mM) is added to the buffer to prevent bacterial growth.
The above procedure can be used for purification and simultaneous concentration of monoclonal IgG antibodies from culture supernatants, as well as for purification of monoclonal antibodies from ascites.
Attention should be paid to the fact that affinity chromatography on SPA-Sepharose cannot be used to identify the class of monoclonal antibodies. In fact, mouse IgG subclasses show a given level of heterogeneity as for affinity of SpA binding: for instance, an antibody of the IgG1 subclass will not be eluted at pH 6 where the bulk of serum IgG1 are eluted, but at pH 4.5, where some IgG1 are eluted with the bulk of IgG2a and IgG3.