Application Note

Antibody Purification

Using the Econo-Pac® Protein A Cartridge and the Econo System

Data generated by Bio-Rad Laboratories Research and Development Group, Hercules, CA USA

Summary

The Econo-Pac protein A cartridge and the Econo System can be used to purify mouse monoclonal antibody from ascites fluid and hybridoma tissue culture supernatant. For these applications, the MAPS® II buffers are recommended, as they augment the binding of low-affinity antibodies to the protein A matrix. Other buffer systems can be used with the Econo-Pac protein A cartridge, but binding capacities of the protein A support must be determined empirically.

Supporting Data

Ascites fluid containing approximately 600 µg/ml IgG_3 was diluted into binding buffer and filtered with a 0.45 µm filter prior to injection onto the Econo System. Figure 1 shows the separation, and Figure 2 demonstrates the specificity of separation.

Fractions were analyzed by SDS-PAGE under reducing conditions, and subsequently stained with Coomassie blue stain. No immunoglobulin heavy and light chains were detected in the flow-through (lane 3), and the eluate appeared to be highly purified immunoglobulin (lane 4).

Hybridoma tissue culture supernatant containing approximately 30–40 µg/ml antibody was also purified with this system. This application was completely automated using the Automated Econo System.

In Figure 3, IgG_{2b} is clearly resolved from other components in the mixture. Fractions were again analyzed by SDS-PAGE under reducing conditions (Figure 4). No immunoglobulin heavy and light chains were detected in the flow-through (lane 3), and eluate appeared to be highly purified immunoglobulin (lane 4).

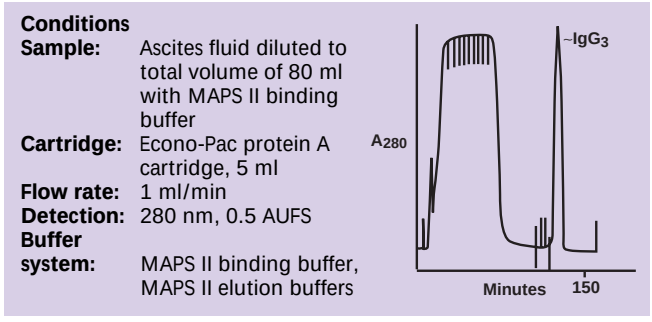

Fig. 1. Mouse monoclonal IgG_3 purified from ascites fluid using the Econo-Pac protein A cartridge.

Fig. 2. SDS-PAGE analysis of pooled fractions from ascites purification. Fractions were run on a 12% Mini-PROTEAN II ready gel and stained with Coomassie® blue stain. Lane 1: Bio-Rad's Low MW standards. Lane 2: Ascites fluid. Lane 3: Unbound fraction. Lane 4: Eluate, purified IgG_3.

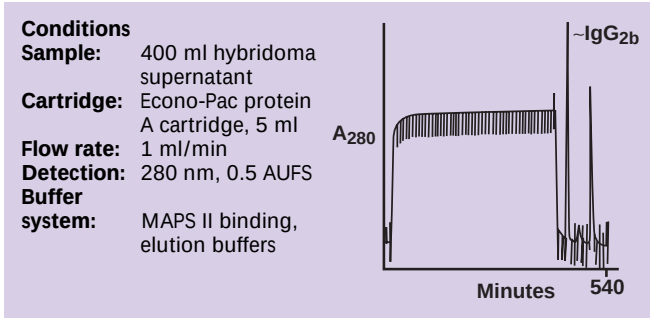

Fig. 3. Mouse monoclonal IgG_{2b} purified from hybridoma supernatant using the Econo-Pac protein A cartridge.

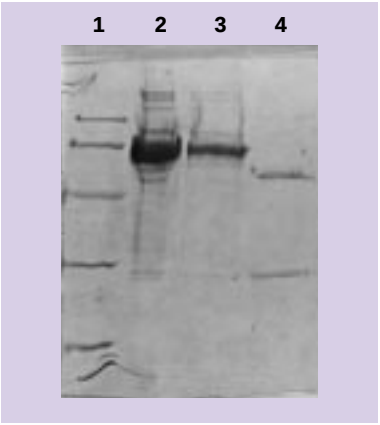

Fig. 4. SDS-PAGE analysis of pooled fractions from hybridoma supernatant purification. Fractions were run on a 12% Mini-PROTEAN II ready gel and stained with Coomassie blue stain. Lane 1: Bio-Rad's Low MW standards. Lane 2: Hybridoma supernatant. Lane 3: Unbound fraction. Lane 4: Eluate, purified IgG_{2b}.

Procedure

For ascites fluid

1. Dilute ascites fluid 1:3 in MAPS II binding buffer and filter using 0.45 µm filter.
2. Equilibrate Econo-Pac protein A cartridge at 1 ml/min with MAPS II binding buffer for 20 minutes. (Note: this procedure can also be automated using the Automated Econo System.)
3. Load sample and continue with a 40 ml binding buffer wash.
4. Elute immunoglobulin with 30 ml MAPS II elution buffer. Neutralize the antibody fractions with 1 M Tris, pH 8.8, since prolonged exposure of the purified antibody to acidic pH should be avoided.
5. Regenerate the column with 50% methanol for 30 minutes.
6. Sanitize the cartridge with 1.0 M NaOH for 30 minutes.
7. Wash with MAPS II binding buffer for 20 minutes to return the column to starting conditions.

For tissue culture supernatant

1. Add 31.4 g MAPS II binding buffer salts to every 100 ml of tissue culture supernatant, so as to avoid further dilution of sample. Pass sample through 0.45 µm filter.
2. Set flow rate to 1 ml/min.
3. Wash with MAPS II binding buffer for 10 minutes.
4. Load the sample for 400 minutes. (More thorough binding of antibodies can be accomplished by either loading sample at a slow flow rate or by recycling sample over the column several times. For this example, the former method was chosen.) Begin collecting fractions of approximately 7.5 ml.
5. Wash column with MAPS II binding buffer for 20 minutes.
6. Elute immunoglobulin with 30 ml MAPS II elution buffer. Neutralize the antibody fractions with 1 M Tris, pH 8.8, since prolonged exposure of the purified antibody to acidic pH should be avoided.
7. Regenerate the column with 50% methanol for 30 minutes.
8. Sanitize the cartridge with 1.0 M NaOH for 30 minutes.
9. Wash with MAPS II binding buffer for 20 minutes to return the column to starting conditions.

Ordering Information

Catalog Number	Product Description
732-0091	**Econo-Pac Protein A Cartridge,** 5 ml
732-0093	**Econo-Pac Protein A Cartridge,** 1 ml
153-6164	**Affi-Prep Protein A MAPS II Buffers,** contains 471 g binding buffer (1,500 ml) and 25 g elution buffer (1,100 ml)

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Printed in USA

Bulletin 1836 US/EG 95-0063 0395