Econo-Pac®
Protein A Kit
Protein A
Columns

Instruction
Manual

Catalog Number
732-2020
732-2022

For Technical Service

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in the U.S. Call **1-800-4BIORAD**

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Section 1
Econo-Pac Protein A Kit

1.1 Introduction

The Econo-Pac protein A kit is based upon Bio-Rad's Affi-Gel® protein A MAPS® II methods and contins everything necessary to purify monoclonal antibodies from ascites or serum. Affi-Gel protein A agarose consists of purified protein A coupled to crosslinked agarose beads via chemically stable amide bonds. The Econo-Pac protein A column contains 2 ml of Affi-Gel protein A with a total capacity of 10-14 mg mouse IgG_1 when used with the MAPS II buffers (included).

The Econo-Pac protein A kit provides a simple and convenient method for the purification of all IgG subclasses, including IgG_1, from ascites fluid. Sample preparation has been simplified by the use of the Econo-Pac 10DG desalting columns. Binding, elution, and regenerations steps can be completed with only one column volume of buffer per step, making the total procedure fast and easy.

1.2 Kit Components

Econo-Pac protein A column	One Econo-Pac column packed with 2 ml of Affi-Gel protein A agarose.
Econo-Pac 10DG desalting columns	Four Econo-Pac 10DG desalting columns.
Binding buffer	One bottle (471 g) of buffer solids, reconstitution volume = 1,500 ml.
Elution buffer	One bottle (25 g) of buffer solids, reconstitution volume = 1,000 ml.
Regeneration buffer	One bottle containing 400 ml. CAUTION: Contains methanol. DO NOT MOUTH PIPETTE.
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The Econo-Pac protein A kit contains a sufficient quantity of reagents to purify approximately 300 mg of mouse IgG_1.

1.3 Additional Items Required

Column rack	The Econo-Pac 10 (12 place) acrylic rack is ideal for holding the Econo-Pac columns. Other benchtop or lattice mount racks may also be used.
Test tubes	General purpose tubes for fraction collection are recommended.
pH meter	A pH meter is required to check the pH of the binding and elution buffers after reconstitution.
Mixer	Standard laboratory magnetic stirrer and bar for buffer mixing.
Balance	Standard laboratory scale for weighing out buffer solids.
Filters	0.22 micron filters for buffer preparation.

1.4 Buffer Preparation

Binding Buffer Preparation

The binding buffer is supplied as a premixed, preweighed solid. Reconstitution and filtration are required prior to use. Dissolve 31.4 g binding buffer solids in distilled, deionized water for a final volume of 100 ml. (Use the full 471 g for 1,500 ml.) Stir for 10 minutes. Filter through a 0.22 µm nylon filter and check the pH. The pH should be 9.0 ± 0.2. If the pH is not in this range, adjust the pH with 10 N NaOH or 6 N HC1. Store buffer solids at room temperature. Stoer reconstituted buffer at 4 °C. If desired, sodium azide may be added to 0.05% (w/v).

Elution Buffer Preparation

The elution buffer is supplied as a preweighed, premixed solid. Reconstitution and filtration are required prior to use. These salts are hygroscopic. Any material in clumps should be broken up before you weight the solids. Dissolve 2.2 g in distilled, deionized water for a final volume of 100 ml (use the full 25 g for 1,100 ml). Stir for 10 minutes. Filter through a 0.22 µm filter and check the pH. The pH should be 3.0 ± 0.2. If the pH is not in this range, adjust the pH with 10 N NaOH or 6 N HC1. Store buffer solids at room temperature. Reconstituted buffer should be stored at 4 °C, and if desired, Thimerosal bacteriostat may be added to a final concentration of one part per 10,000. The use of sodium azide in low pH buffers is hazardous.

1.5 Sample Preparation

Sample preparation is simplified by using the Econo-Pac 10DG columns. Each Econo-Pac 10DG desalting column can process up to 3 milliliters of ascites fluid and the columns can be re-used. Two Econo-Pac 10DG columns should be reserved for buffer exchange at the end of the protein A purification protocol. It is also recommended that one Econo-Pac 10DG desalting column be used for each (different) ascites to avoid cross contamination.

To prepare up to 3 ml of ascites for purification on the Affi-Gel protein a column:

1. Discard the buffer above the top frit of one Econo-Pac 10DG column.
2. Add 20 ml of binding buffer to the column (fill to the top), and snap off the bottom tip to start the column flowing.
3. Allow the buffer to drain to the top frit. The column will not run dry. Flow will stop when the buffer level reaches the top frit.
4. Add 3.0 ml of ascites fluid to the column. If the sample is less than 3.0 ml, add buffer to reach a total sample volume of 3.0 ml.
5. Allow the sample to completely run into the column. Discard the first 3.0 ml eluted.
6. Add 4.0 ml of binding buffer to elute the ascites, while collecting the 4.0 ml fraction from the column.
7. Wash the Econo-Pac 10DG column with 20 ml of binding buffer if the column is going to be used again right away, or wash the column with 20 ml of water containing 0.02% sodium azide for storage.

1.6 Standard Mouse IgG_1 Purification Procedure

1. Discard the buffer above the top frit of the Econo-Pac protein A column.
2. Equilibrate the column with 10 ml binding buffer. Allow the buffer to drain to the top frit. The column will not run dry. After equilibration, the pH of the column effluent should be equal to the pH of the binding buffer (pH 9.0).
3. Apply the prepared sample to the column. Maximum recommended sample volume is 2.0 ml ascites fluid.

4. Wash the column with 20 ml binding buffer.
5. Elute the IgG with 10 ml of elution buffer. Elute the column with an additional 20 ml of buffer to insure total removal IgG. In most cases, the majority of the IgG will elute in the first 10 ml.
6. Wash the column with 10 ml of regeneration buffer, followed by 10 ml of PBS containing 0.02% sodium azide for storage.
7. Set up an unused Econo-Pac 10DG column previously equilibrated with 20 ml of PBS or other buffer suitable for storage of immunoglobulin.
8. Apply 3 ml of the IgG-containing fraction collected in step 5 to the prepared Econo-Pac 10DG column. Discard the first 3.0 ml eluted.
9. Add 4.0 ml of appropriate buffer (see step 7) to the Econo-Pac 10DG column and collect the 4.0 ml fraction from the column. This fraction contains the protein A purified IgG. For a more precise collection method, elute the antibody with 8.0 ml of a suitable buffer and collect 1.0 ml fractions. Analyze the fractions to determine which fractions to pool.
10. Repeat until the entire IgG-containing fraction has been buffer exchanged.
11. The Econo-Pac 10DG column should then be washed with 20 ml of deionized water. Store at room temperature with 0.02% sodium azide for re-use.

1.7 Answers to Common Questions

1. **Sensitivity of antibodies to low pH:**
   There are a few antibodies which are inactivated by low pH. Inactivation can be avoided by collecting the eluted fractions into a concentrated neutral buffer or buffer exchanging the fraction using an Econo-Pac 10DG column. In cases of extreme sensitivity, many immunoglobulins can be eluted at pH 4-6 by adjusting the pH of the elution buffer with 10 N NaOH.

2. **Expected flow rate:**
   0.5-1.5 ml/min.

3. **Column regeneration:**
   Regenerating the column after every use will increase the lifetime of the gel and help eliminate cross-over contamination of IgG from one run to the next.

4. **Shelf life to the Econo-Pac protein A kit:**
   The shelf life of the Econo-Pac protein A column is 1 year at 4 °C. The unreconstituted buffers are good for at least 1 year when stored at room temperature. Reconstituted buffers can be stored at 4 °C for approximately 2 weeks.

5. **Purification of IgG from serum or tissue culture fluids:**
   In addition to ascites, serum and tissue culture samples are suitable as samples for the Econo-Pac protein A kit. Serum samples will require the same sample preparation and purification procedures as ascites samples (as outlined in this manual).

   Tissue culture fluids should be concentrated to approximately 5 mg immunoglobulin/ml and then processed on an Econo-Pac 10DG desalting column as described in this manual (or diluted one to one with binding buffer). The purification procedure for the concentrated tissue culture fluid will then be identical to the ascites procedure.

6. **Purification of IgG other than mouse:**
   Human and rabbit IgG from serum (Bio-Radiations No. 53) have been purified successfully with Affi-Gel protein A gel. The protocol in this manual can be used without modification.

7. **Purification of IgM with the Econo-Pac protein A kit:**
   Protein A will bind approximately 50% of all IgMs. The purification of IgM using the Econo-Pac protein A kit will therefore vary with each particular IgM sample.

Section 2
Econo-Pac Protein A Columns

2.1 Introduction

Bio-Rad's Econo-Pac protein A columns are Econo-Pac 10 chromatography columns packed with 2 ml of Affi-Gel protein A gel with an upper frit above the gel bed. Affi-Gel protein A consists of purified protein A coupled to crosslinked agarose beads via stable amide bonds. When used with the patented MAPS II buffer system, each column has a capacity of 10-14 mg mouse IgG_1.

The Econo-Pac protein A columns provide a rapid and convenient way to purify monoclonal antibodies from small amounts of ascites fluid. Binding, elution, and regeneration steps can be com-

pleted with only one column volume of buffer per step, making the total procedure fast and easy.

2.2 Buffer Considerations

The affinity of IgG for protein A is not the same for all species. Most mouse IgG_1 immunoglobulins have low affinity for protein A. For this reason, the patented MAPS II buffer system has been developed to optimize binding and recovery of mouse IgG_1.[1] When Affi-Gel protein A is combined with the specially optimized MAPS II buffer system, protein A capacity for mouse IgG_1 from ascites fluid is 6-8 mg/ml gel. (This capacity is 8-10 times higher than that obtained with published methods.[1,2]) Using the MAPS II buffers, the Econo-Pac protein A columns therefore have a mouse IgG_1 capacity of 10-14 mg per column.

An alternative binding buffer is 10 mM sodium phosphate, 0.15 M sodium chloride, pH 8.2. A typical elution buffer is 0.1 M sodium citrate, and a suitable regeneration buffer is 1.5 M sodium thiocyanate.

2.3 Sample Preparation

Bio-Rad's Econo-Pac 10DG disposable desalting columns are recommended for easy ascites or serum preparation with minimal sample dilution. Each desalting column can prepare up to 3 milliliters of raw ascites. The ascites sample is collected in 4 milliliters of binding buffer, so sample dilution is minimized. An alternative is to dilute ascites sample one to one with binding buffer, and then adjust the pH to that of the binding buffer.

If your sample is tissue culture supernatant, it should be concentrated to approximately 5 mg immunoglobulin/ml and then diluted one to one with binding buffer.

2.4 Standard Mouse IgG Purification Procedure

1. Discard the buffer above the top frit of the Econo-Pac protein A column.
2. Equilibrate the column with 10 ml binding buffer. Allow the buffer to drain to the top frit. The column will not run dry.
3. Apply prepared sample to the column. See "Column Performance" for recommended sample volumes.
4. Wash the column with 20 ml binding buffer.

5. Elute the IgG with 10 ml of elution buffer. Elute the column with an additional 20 ml of buffer to insure total removal of IgG. In most cases, the majority of the IgG will elute in the first 10 ml. Prolonged exposure to acid pH should be avoided. Neutralize immediately after elution. The Econo-Pac 10DG desalting columns can be used to rapidly neutralize, desalt and buffer exchange the eluate into a suitable buffer (e.g., PBS). [Note: the use of the Econo-Pac 10 DG desalting columns effectively eliminates the need for lengthy dialysis procedures required when samples are to be analyzed by SDS-PAGE.]
6. Wash the column with 10 ml of regeneration buffer.
7. Wash the column with 10 ml of binding buffer for the next chromatography cycle or store in PBS containing 0.05% sodium azide at 4 °C.

2.5 Column Performance

Packed gel	Affi-Gel protein A
Bed volume	2.0 ml
Reservoir volume	30 ml
Colume capacity	10-14 mg IgG_1
Recommended sample volume	Maximum 2.0 ml ascites or serum
Packing buffer	10 mM sodium phosphate, 150 mM NaC1, pH 7.0, with 0.02% sodium azide
Flow rate range	0.5-1.5 ml/min
Column material	Polypropylene
Frit material	Nominal 35 µm porous, polyethylene
pH range	pH 2-11
Recommended storage	4 °C, with PBS containing 0.02% sodium azide

Section 3
References

1. Ey, P. L., Prowse, S. J. and Jenkins, C. R., *Immunochemistry*, **15**, 429 (1978).
2. Begbee, W. L., Vanderlaan, M., Fong, S. S. N. and Jensen, R. M., *Mol. Immunol.*, **20**, 153 (1983).

Section 4
Ordering Information

Catalog Number	Product Description
732-2020	**Econo-Pac Protein A Kit,** includes one 2 ml Affi-Gel protein A prepacked Econo-Pac column, 4 Econo-Pac 10 DG desalting columns, binding, elution, and regeneration buffers.
732-2022	**Econo-Pac Protein A Columns,** 5
732-2010	**Econo-Pac 10 DG Desalting Columns,** 30
732-1015	**Econo-Pac 10 Rack,** 12 place

Other Affi-Gel Protein A Products from Bio-Rad:

153-6153	**Affi-Gel Protein A Agarose,** 5 ml
153-6154	**Affi-Gel Protein A Agarose,** 50 ml
153-6159	**Affi-Gel Protein A MAPS II Kit,** includes 5 ml Affi-Gel protein A, binding, elution and regeneration buffers, and 1 x 10 cm Econo-Column chromatography column.
153-6160	**Affi-Gel Protein A MAPS II Buffers,** includes binding, elution, and regeneration buffers.
153-6161	**Affi-Gel Protein A MAPS II Binding Buffer,** 5 liters
153-6162	**Affi-Gel Protein A MAPS II Elution Buffer,** 5 liters
153-6163	**Affi-Gel Protein A MAPS II Regeneration Buffer,** 5 liters

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