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# **Econo-Pac<sup>®</sup> Serum IgG Purification Kit and Econo-Pac Serum IgG Purification Columns Instruction Manual**

**Catalog Numbers  
732-3037 and  
732-2026**

For Technical Service  
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in the U.S. Call **1-800-4BIORAD**  
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**BIO-RAD**



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# Section 1

## Econo-Pac Serum IgG Purification Kit

### Catalog Number 732-3037

#### 1.1 Introduction

The Econo-Pac serum IgG purification kit provides everything necessary for fast and convenient purification of IgG from serum. Serum sample preparation is greatly simplified by using the Econo-Pac 10DG desalting columns, which eliminate the need for lengthy membrane dialysis procedures before chromatography. Prepacked DEAE Affi-Gel® blue columns allow the purification of IgG from other serum proteins and plasminogen. The IgG fraction obtained will have some residual contamination from transferrin. Buffers are provided pre-mixed and preweighed for easy preparation.

#### 1.2 Kit Components

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|                                                 |                                                                                                                                                                        |
|-------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Econo-Pac serum IgG purification columns</b> | Five Econo-Pac columns packed with 10 ml of DEAE Affi-Gel blue gel                                                                                                     |
| <b>Econo-Pac 10DG desalting columns</b>         | Five Econo-Pac 10DG desalting columns.                                                                                                                                 |
| <b>Human application buffer</b>                 | One bottle (13 g) of buffer solids, reconstitution volume = 3,000 ml                                                                                                   |
| <b>Rabbit application buffer</b>                | One bottle (13 g) of buffer solids, reconstitution volume = 3,000 ml.                                                                                                  |
| <b>Regeneration buffer</b>                      | One bottle (121 g) of buffer solids, reconstitution volume = 1,000 ml. <b>Caution:</b> Contains sodium thiocyanate. Avoid skin contact. Avoid breathing dust or fumes. |

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#### 1.3 Additional Items Required But Not Provided

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|                    |                                                                                                                                              |
|--------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Column rack</b> | 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. |
| <b>Test tubes</b>  | General purpose tubes for fraction collection are recommended.                                                                               |
| <b>pH meter</b>    | A pH meter is required to check the pH of the human and rabbit buffers after reconstitution.                                                 |
| <b>Mixer</b>       | Standard laboratory magnetic stirrer and bar for buffer mixing.                                                                              |
| <b>Balance</b>     | Standard laboratory scale for weighing out buffer solids.                                                                                    |
| <b>Filters</b>     | 0.45 micron filters for buffer preparation.                                                                                                  |

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## 1.4 Buffer Preparation

### Human Application Buffer Preparation

The human buffer is supplied as a premixed, preweighed solid. Reconstitution and filtration are required prior to use. Dissolve 1.3 grams human buffer solids per 300 ml (final volume) distilled, deionized water. (Use the full 13 grams for 3 liters final volume.) Filter through a 0.45  $\mu\text{m}$  filter and check the pH. The pH should be  $8.0 \pm 0.2$ . If the pH is not in this range, adjust the pH with 10 N KOH or 6 N HCl. Store buffer solids at room temperature. Store reconstituted buffer at 4 °C. If desired, sodium azide may be added to 0.05% (w/v).

### Rabbit Application Buffer Preparation

The rabbit buffer is supplied as a premixed, preweighed solid. Reconstitution and filtration are required prior to use. Dissolve 1.3 grams rabbit buffer solids per 300 ml (final volume) distilled, deionized water. (Use the full 13 grams for 3 liters final volume.) Filter through a 0.45  $\mu\text{m}$  filter and check the pH. The pH should be  $8.0 \pm 0.2$ . If the pH is not in this range, adjust the pH with 10 N NaOH or 6 N HCl. Store buffer solids at room temperature. Store reconstituted buffer at 4 °C. If desired, sodium azide may be added to 0.05% (w/v).

### Regeneration Buffer Preparation

The regeneration buffer is supplied as a premixed, preweighed solid. Reconstitution and filtration are required prior to use. Dissolve 12.2 grams per 100 ml (final volume) distilled, deionized water. (Use the full 121 grams for 1 liter final volume.) Filter through a 0.45  $\mu\text{m}$  filter. No pH adjustment is necessary. Store buffer solids at room temperature. Store reconstituted buffer at 4 °C.

## 1.5 Sample Preparation

Sample preparation is simplified by using the Econo-Pac 10DG columns. Each Econo-Pac 10DG column can process up to 3 milliliters of serum or ascites fluid per cycle. The columns can be re-used. It is recommended that one Econo-Pac 10DG column be used for each (different) serum to avoid cross contamination.

To prepare up to 3 ml of serum for purification on the serum IgG purification columns:

1. Discard the buffer above the top frit of one Econo-Pac 10DG column.
2. Add 20 ml of application buffer (either human or rabbit 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 serum to the column. If the serum sample is less than 3.0 ml, add application 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 the application buffer to elute the serum, while collecting the 4.0 ml fraction from the column.
7. Wash the Econo-Pac 10DG column with 20 ml of the application buffer if the column is to be used again immediately. If the column is to be stored, wash with 20 ml of water containing 0.02% sodium azide.

## 1.6 Standard Serum IgG purification

1. Discard the buffer above the top frit of an Econo-Pac serum IgG purification column and snap off the bottom tip.
2. Prewash for first time use only: Wash the column with 40 ml of regeneration buffer\* (fill the column twice), follow by 40 ml of the appropriate application buffer (human or rabbit). Allow the buffer to drain to the top of the frit. The column will not run dry. Proceed with step 4.

**\*Caution:** Contains sodium thiocyanate. Avoid skin contact. Avoid breathing dust or fumes.

[If the column has already been prewashed proceed directly to step 3.]

3. Equilibrate the column with 30 ml of application buffer.
4. Apply prepared sample to the column. See label on column package for exact column serum capacity.
5. Elute the IgG with 20 ml of application buffer. For a more precise collection method, collect fractions of volumes approximately equivalent to that of the sample applied. Determine the absorbance at 280 ml of each fraction using a spectrophotometer and combine effluent tubes containing the unbound protein peak.
6. An optional step: Most of the bound albumin can be eluted by washing the column with application buffer containing 1.4 M NaCl.
7. Regardless of whether or not step 6 was carried out, regenerate the column with 20 ml of regeneration buffer.
8. Wash the column with 30 ml of application buffer and continue with step 4 for the next chromatography cycle, or store the column at 4 °C in application buffer containing 0.02% sodium azide.

## 1.7 Answers to Common Questions

1. **How do I purify polyclonal IgG from sheep or goat serum?**

The Econo-Pac serum IgG purification kit can be used for goat and sheep serum. In these cases, the rabbit buffer will be suitable.

2. **What is the purpose of the regeneration buffer wash the first time the column is used?**

For new preparations of gel, this initial wash will elute residual blue dye which might otherwise be eluted in serum protein fractions.

3. **What are typical flow rates?**

0.8-1.5 ml/min.

4. **What is the shelf life of the Econo-Pac serum IgG purification kit?**

The shelf life of the Econo-Pac serum IgG purification kit is 1 year at 4 °C. The unreconstituted buffers are good for at least 1 year when stored at room temperature in a tightly sealed bottle.

## Section 2

# Econo-Pac Serum IgG Purification Columns

### Catalog Number 732-2026

## 2.1 Introduction

Econo-Pac serum IgG purification columns are Econo-Pac 10 chromatography columns packed with 10 ml of DEAE Affi-Gel blue gel with an upper frit above the gel bed. DEAE Affi-Gel blue gel consists of Cibacron® Blue F3GA dye coupled to specially prepared DEAE Bio-Gel® A gel. Chromatography on DEAE Affi-Gel blue agarose gel produces a purified IgG fraction with only residual contamination from transferrin. After the IgG has been eluted, additional serum fractions can be obtained from the column by elution with an ionic strength gradient.

The Econo-Pac serum IgG purification columns provide a rapid and convenient way to purify IgG from serum.

## 2.2 Buffer Consideration

### Prewash

When using an Econo-Pac serum IgG purification column for the first time, a prewash is required to elute from the column residual dye which might otherwise be eluted in serum protein fractions. Wash the column with 40 ml (fill the column twice) of either:

- 1.5 M sodium thiocyanate, or
- 0.10 M acetic acid, pH 3.0, containing 1.4 M NaCl and 40% (v/v) isopropanol.

## Application Buffers

Buffer ionic strength is a critical parameter in DEAE Affi-Gel blue chromatography. Small variations in salt concentration and pH significantly effect sample purity and/or sample recovery. The appropriate buffer composition for human and rabbit serum chromatography has been determined in our laboratories as follows:

| <b>Serum</b> | <b>Buffer</b>                                    |
|--------------|--------------------------------------------------|
| Rabbit       | 0.02 M Tris-HCl, pH 8.0, containing 0.028 M NaCl |
| Human        | 0.02 M K <sub>2</sub> HPO <sub>4</sub> , pH 8.0  |

The Econo-Pac serum IgG purification columns can also be used to purify serum IgG from other species. For species such as goat, rat, or sheep, the rabbit buffer is suitable.

### Regeneration buffer

The columns should be regenerated after each chromatography run. This will insure removal of any bound proteins and prevent cross-over contamination from one run to the next. Suitable regeneration buffers include 1.5 M sodium thiocyanate or 2 M guanidine HCl in the application buffer.

## 2.3 Sample Preparation

Bio-Rad's Econo-Pac 10DG desalting columns are recommended for serum or ascites preparation. Each desalting column can prepare up to 3 milliliters of serum. The serum sample is then collected in 4 milliliters of application buffer, so sample dilution is minimal. The whole procedure takes approximately 30 minutes. Alternatively, dialysis of the serum sample against the appropriate application buffer may be used before chromatography to reduce the serum salt content and adjust the pH.

## 2.4 Standard Serum IgG Purification Procedure

1. Discard the buffer above the top frit of an Econo-Pac serum IgG purification column and snap off the bottom tip.
2. Prewash the first time use only: Wash the column with 40 ml of prewash buffer (fill the column twice), followed by 40 ml of the appropriate application buffer (human or rabbit). Allow the buffer to drain to the top of the frit. The column will not run dry. Proceed to step 4.

[If the column has already been prewashed, begin the procedure with step 3.]

3. Equilibrate the column with 30 ml of application buffer.
4. Apply prepared sample to the column. See label on column package for exact column serum capacity.

5. Elute the IgG with 20 ml of application buffer. For a more precise collection method, collect fractions of volume approximately equivalent to that of the sample applied. Determine the absorbance at 280 nm of each fraction using a spectrophotometer and combine effluent tubes containing the unbound protein peak.

[For the isolation of other serum proteins, the column may be eluted with a gradient of increasing NaCl concentration. A final saline concentration of 0.5 M will elute most serum proteins other than albumin.]

6. An optional step: Most of the bound albumin can be eluted by washing the column with application buffer containing 1.4 M NaCl.
7. Regardless of whether or not step 6 is carried out, regenerate the column with 20 ml of regeneration buffer.
8. Wash the column with 30 ml of application buffer and continue with step 4 for the next chromatography cycle, or store the column at 4 °C in application buffer containing 0.02% sodium azide.

## 2.5 Column Performance

|                              |                                                                      |
|------------------------------|----------------------------------------------------------------------|
| <b>Packed gel</b>            | DEAE Affi-Gel blue gel                                               |
| <b>Bed volume</b>            | 10 ml of packed gel                                                  |
| <b>Total column volume</b>   | 30 ml                                                                |
| <b>Column serum capacity</b> | See label on column package for exact column capacity                |
| <b>Packing buffer</b>        | 10 mM sodium phosphate, 150 mM NaCl, pH 7.0, with 0.02% sodium azide |
| <b>Flow rate range</b>       | 0.8-1.5 ml/min                                                       |
| <b>Column material</b>       | Polypropylene                                                        |
| <b>Frit material</b>         | Nominal 35 µm porous, polyethylene                                   |
| <b>Recommended storage</b>   | 4 °C, in application buffer containing 0.02% sodium azide.           |

## Section 3 Product Information

### Catalog

#### Number Product Description

|          |                                                                                                                                                                                                       |
|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 732-2027 | <b>Econo-Pac Serum IgG Purification Kit</b> , includes Econo-Pac serum IgG purification columns (5), Econo-Pac 10DG desalting columns (5), human and rabbit application buffers, regeneration buffer. |
| 732-2026 | <b>Econo-Pac Serum IgG Purification Columns, 5</b>                                                                                                                                                    |
| 153-7307 | <b>DEAE Affi-Gel Blue Gel</b> , 100 ml                                                                                                                                                                |
| 732-2010 | <b>Econo-Pac 10DG Desalting Columns</b> , 30 columns                                                                                                                                                  |
| 732-1015 | <b>Econo-Pac 10 Rack</b> , 12 place                                                                                                                                                                   |



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