INSTRUCTIONS

Binding and Elution Buffers

0411.6

Number	Description
21001	**Protein A IgG Binding Buffer,** 1L, pH 8.0; contains EDTA as a preservative
21007	**Protein A IgG Binding Buffer,** 3.75L, pH 8.0; contains EDTA as a preservative
21011	**Protein G IgG Binding Buffer,** 3.75L, pH 5.0; with 0.02% sodium azide
21019	**Protein G IgG Binding Buffer,** 1L, pH 5.0; with 0.02% sodium azide
54200	**Protein A/G IgG Binding Buffer,** 240mL pH 8.0; contains EDTA as a preservative
21004	**IgG Elution Buffer,** 1L, pH 2.8
21009	**IgG Elution Buffer,** 3.75L, pH 2.8
21028	**IgG Elution Buffer,** 1L, pH 2.0
	Note: The binding buffers are processed through a 0.2µm filter. The elution buffer contains primary amines and is purged with nitrogen to exclude oxygen.
	Storage: Upon receipt store buffers at 4°C. Buffers are shipped at ambient temperature.

Introduction

The Thermo Scientific Binding and Elution Buffers are optimized to provide the highest efficiency of IgG binding and recovery when used with various immobilized immunoglobulin-binding proteins.

Note: The Protein A IgG Binding Buffer precipitates in the presence of SDS.

Procedure for Purifying IgG

A. Additional Materials Required

- Immobilized Protein A, Protein G, Protein A/G, or Protein L packed into a gravity-flow column

  Note: While the same elution buffer is effective for all of these immobilized proteins, a different binding buffer is required for optimal binding with each. For Protein L, use phosphate-buffered saline for binding (Product No. 28372).
- Neutralization Buffer: Prepare 1mL of high-ionic strength alkaline buffer such as 1M phosphate or 1M Tris, (pH 7.5-9)
- Thermo Scientific Slide-A-Lyzer Dialysis Cassette or Zeba Spin Desalting Columns (Product No. 43230) for buffer exchange

B. Procedure

1. Equilibrate the buffers and column to room temperature.
2. Dilute serum samples, ascites fluid, or tissue culture supernatant at least 1:1 with binding buffer to ensure the ionic strength and pH are maintained for optimal binding.

   Note: If plasma is used, the sample may appear hazy after adding the binding buffer caused by lipoprotein precipitation. For optimal antibody recoveries, centrifuge the diluted sample at 10,000 × *g* for 20 minutes and apply the supernatant to the equilibrated column.
3. Equilibrate the column with five resin-bed volumes of binding buffer.
4. Apply the diluted sample to the column and allow it to flow completely into the resin.
5. Wash the column with 5-10 resin-bed volumes of binding buffer.

6. Elute antibodies with five resin-bed volumes of elution buffer and collect 0.5-1mL fractions. Immediately adjust eluted fractions to physiologic pH by adding 100µL of Neutralization Buffer to 1mL of eluate. Monitor elution by measuring the absorbance at 280nm or by protein assay such as Thermo Scientific BCA Protein Assay Kit (Product No. 23225).
7. Pool the eluted IgG fractions that contain the highest absorbance values. Use the purified antibodies directly for SDS-PAGE or exchange the buffer to a system compatible with the specific downstream application.
8. Regenerate the affinity column by applying eight resin-bed volumes of Elution Buffer and allowing it to flow through.
9. For storage, wash column with five resin-bed volumes of water containing 0.02% sodium azide. When ~3mL of solution remains above the top disc, replace bottom cap and then the top cap on the column. Store column upright at 4°C.

Elution of Antibody/Antigen Interactions

The IgG Elution Buffer is an effective universal buffer for disrupting antigen:antibody interactions. The most effective elution conditions (pH, ionic strength, chaotrope, or denaturant) for a particular interaction, however, depends on the specific composition of ionic, hydrophobic and hydrogen bonds involved. All elution buffers cause various levels of antibody or antigen functionality loss, limiting the number of times an affinity support can be reused. For more information, see Tech Tip #27: Optimize elution conditions for immunoaffinity purification, which is available from the Pierce website.

Note: This example procedure assumes that an antigen has been immobilized to a beaded resin and is being used in a column format to purify antibody from serum or culture supernatant. Use the same procedure for the converse purification scheme (i.e., immobilized antibody column to purify an antigen) by simply switching all references to antigen and antibody.

1. Equilibrate buffers and column of immobilized antigen to the same temperature (room temperature or 4°C).
2. Prepare antibody sample for binding. Dilute concentrated samples such as serum and ascites fluid with an equal volume of a physiological buffer (i.e., PBS or TBS). Adjust cell culture supernatant and other dilute samples to pH 7.2-7.4 with TBS or PBS. Centrifuge cloudy samples and use only the clear supernatant.
3. Wash and prepare the antigen column by adding five resin-bed volumes of buffer and allowing it to flow through. Discard the flow-through storage buffer.
4. Add the prepared antibody sample to the antigen column and allow it to flow through. If desired, stop the column occasionally or control the flow rate to ensure adequate binding time. Save the flow-through non-bound sample.
5. Wash column by adding 5-10 resin-bed volumes of buffer and allowing it to flow through. If desired, analyze flow-through fractions to determine if washing is complete.
6. Elute the purified antibody by adding 5-10 resin-bed volumes of Elution Buffer and collecting the flow-through in several small fractions. Immediately adjust eluted fractions to physiologic pH by adding 100µL of Neutralization Buffer (i.e., 1M phosphate or 1M Tris; pH 7.5-9) per 1mL of eluate. Fractions confirmed to contain antibody may be pooled, and the other fractions may be discarded.
7. Dialyze or desalt to exchange the purified antibody fractions into a buffer suitable for storage and analysis.

Related Thermo Scientific Products

28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
69576	**Slide-A-Lyzer™ MINI Dialysis Device Kit, 10K MWCO, 0.1mL**
66382	**Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO, 3mL**
89889	**Zeba™ Spin Desalting Columns, 7K MWCO, 2mL,** 5/pkg
89891	**Zeba Spin Desalting Columns, 7K MWCO, 5mL,** 5/pkg
44894	**AminoLink™ Plus Immobilization Kit**
44999	**SulfoLink™ Immobilization Kit for Peptides**
44899	**CarboxyLink™ Immobilization Kit**

General References

Bigbee, W.L., *et al.* (1983). Monoclonal antibodies specific for the M- and N-forms of human glycophorin A. *Mol Immunol* **20:**1353-62.

Ey, P.L., *et al.* (1978). Isolation of pure IgG_1, IgG_{2a}, and IgG_{2b} immunoglobulins from mouse serum using protein A-Sepharose. *Immunochem* **15:**429-36.

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