

Mouse IgG1 Mild Elution Buffer Kit

21033

0707.1

Number	Description
21033	Mouse IgG1 Mild Elution Buffer Kit Kit Contents: Mouse IgG1 Mild Elution Buffer , pH 6.0, 500 ml Protein A IgG Binding Buffer , pH 8.0, 1000 ml IgG Elution Buffer , pH 2.8, 1 L

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Mouse IgG1 Mild Elution Buffer Kit provides for the selective elution of mouse IgG1 antibodies that were bound together with antibodies of other mouse IgG subclasses to immobilized Protein A (i.e., Protein A agarose). Mouse IgGs are some of the most common immunoglobulins used in immunological research. The four major subclasses of mouse IgG are IgG1, IgG2a, IgG2b, and IgG3. Protein A has varying affinity to these subclasses; it binds most weakly to mouse IgG1. This difference in binding affinity to Protein A provides a means to selectively elute mouse IgG1 and thereby purify this one subclass from a sample containing all mouse IgG subclasses.

The Protein A IgG Binding Buffer is optimized to ensure that mouse IgG1 antibodies efficiently bind to Protein A agarose along with other mouse IgG subclasses in a sample. The Mouse IgG1 Mild Elution Buffer is sufficient to dissociate the weakly bound mouse IgG1 antibodies without also eluting other bound IgGs. After the mouse IgG1 antibodies have been eluted and recovered from the Protein A affinity column, the low-pH IgG Elution Buffer can be used to elute the remaining IgGs, thereby regenerating the Protein A column for reuse.

Procedure for Mouse IgG1 Purification

A. Additional Materials Required

- Protein A affinity column (Protein A Agarose or Protein A Column, e.g., Product No. 20356). Use an amount of resin or column size with sufficient binding capacity for the total IgG in the sample
- UV spectrophotometer for evaluating protein content (280 nm) of wash and elution fractions

B. Procedure

1. Equilibrate buffers and Protein A column room temperature.
2. Dilute sample at least 1:1 with the binding buffer. If the solution is cloudy, centrifuge at $10,000 \times g$ for 20 minutes and carefully remove the supernatant for use. Determine the total absorbance (concentration) of the sample at 280 nm.
3. Sequentially remove the top and bottom caps from the column and drain storage solution.
4. Equilibrate column by washing with 5 resin-bed volumes of the Protein A IgG Binding Buffer.
5. Apply prepared sample to the protein A column and allow it to flow through into the resin bed. To ensure adequate binding time, consider capping the column bottom for 30 minutes after each resin-bed volume of sample has entered the column.
6. Wash the column with 5 resin-bed volumes of the Protein A IgG Binding Buffer.

7. Elute the bound mouse IgG1 by applying 5 resin-bed volumes of the Mouse IgG1 Mild Elution Buffer and collecting separate fractions as they emerge (for best results, use a fraction size no greater than half the resin-bed volume; this would result in 10 elution fractions). Measure the absorbance of each fraction at 280 nm to determine which ones contain the eluted mouse IgG1.
8. Elute all remaining IgGs and other bound material by applying 8 resin-bed volumes of the IgG Elution Buffer and collecting separate fractions as they emerge. To save these non-IgG1 antibodies, neutralize the collected low-pH fractions by adding 50 µl of 1.0 M Tris•HCl per 1 ml volume of each fraction. Measure the absorbance of each fraction at 280 nm to determine which ones contain the eluted mouse IgG1.
9. The column can be conditioned for reuse by washing it with an additional 4 resin-bed volumes of the IgG Elution Buffer, followed by 4 resin-bed volumes of water or PBS containing 0.05% sodium azide solution. Cap the column when a head of buffer remains above the resin bed. Store column upright at 4°C.
10. Dialyze or desalt to exchange the purified antibody fractions into a buffer suitable for storage and analysis.

Related Products

21001	Protein A IgG Binding Buffer , 1 L, pH 8.0; contains EDTA as a preservative
21007	Protein A IgG Binding Buffer , 3.75 L, pH 8.0; contains EDTA as a preservative
21011	Protein G IgG Binding Buffer , 3.75 L, pH 5.0; with 0.02% sodium azide
21019	Protein G IgG Binding Buffer , 1 L, pH 5.0; with 0.02% sodium azide
54200	Protein A/G IgG Binding Buffer , 240 ml pH 8.0; contains EDTA as a preservative
21004	IgG Elution Buffer , 1 L, pH 2.8
21009	IgG Elution Buffer , 3.75 L, pH 2.8
28372	BupH™ Phosphate Buffered Saline Packs , 40 pack, each pack yields 500 ml
69576	Slide-A-Lyzer MINI Dialysis Unit Kit , for 10-100 µl sample volumes, 10 units plus float
66382, 66807	Slide-A-Lyzer Dialysis Cassette Kits , for 0.5-3 ml and 3-12 ml sample volumes, respectively
89889	Zeba™ Desalt Spin Columns 2 ml , 5 × 2 ml columns, for desalting 200-700 µl samples
89891	Zeba™ Desalt Spin Columns 5 ml , 5 × 5 ml columns, for desalting 500-2,000 µl samples
37501	Monoclonal Antibody Isotyping Kit I (HRP/ABTS)
37502	Monoclonal Antibody Isotyping Kit II. (AP/PNPP)

References

Hermanson, G.T., Mallia, K.A., Smith, P.K. (1992). Immobilized Affinity Ligand Techniques. Academic Press. (Available as Product No. 22230)

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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