INSTRUCTIONS

ImmunoPure® Immobilized Mannan Binding Protein

22212

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>22212</td>
<td>ImmunoPure® Immobilized Mannan Binding Protein, 10 ml settled gel</td>
</tr>
<tr>
<td></td>
<td>Support: 4% beaded agarose</td>
</tr>
<tr>
<td></td>
<td>Capacity: At least 1.5 mg of mouse IgM with &gt;90% purity isolated from a single pass of 0.5 ml ascites diluted with 0.5 ml of ImmunoPure® IgG Binding Buffer using a 5 ml packed column</td>
</tr>
<tr>
<td></td>
<td>Supplied: 10 ml of gel in 10 ml of ImmunoPure® IgM Binding Buffer (20 ml total volume)</td>
</tr>
</tbody>
</table>

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

Introduction

ImmunoPure® Immobilized Mannan Binding Protein combined with an optimized buffer system enables purification of mouse IgM from ascites. Mannan Binding Protein (MBP) is a mannose and N-acetylglucosamine specific lectin present in mammalian sera that is capable of initiating carbohydrate-mediated complement activation. MBP consists of 18 identical subunits, each with molecular mass of approximately 31 kDa. MBP covalently attached to an agarose support produces an excellent tool for affinity purification of IgM.

ImmunoPure® Immobilized MBP is most effective for purifying mouse IgM from ascites. Purified IgM can be obtained from a single pass over the affinity column. Human IgM also will bind to the support, but with slightly lower capacity yielding a product at least 88% pure as assessed by HPLC. Purification of IgM from other species and mouse serum has not yet been optimized. Purification of IgM is temperature and calcium dependent. Binding and washing steps are performed at 4°C in a buffer that contains calcium chloride. Elution is achieved at room temperature in a buffer that contains EDTA and devoid of calcium chloride. The simple protocol is easy to use and yields 90% pure mouse IgM from ascites. Immobilized MBP can be regenerated at least 10 times with no apparent loss of binding capacity.

Additional Materials Required

- Binding Buffer: 10 mM Tris, 1.25 M NaCl, 20 mM CaCl₂; pH 7.4 or ImmunoPure® IgM Binding Buffer (Product No. 21016)
- Elution Buffer: 10 mM Tris, 1.25 M NaCl, 2 mM EDTA; pH 7.4 or ImmunoPure® IgM Elution Buffer (Product No. 21017)
- Disposable column such as the Disposable Polypropylene Columns for 1.0-5.0 ml gel-bed volumes (Product No. 29922) or the Disposable Column Trial Pack (Product No.29925) that contains two each of three column sizes (i.e., 0.5-2.0 ml, 1.0-5.0 ml and 2.0-10.0 ml gel-bed volumes)

Note: For spin-column formats, use Handee™ Mini-Spin Columns and Accessories (Product No. 69705).

Sample Preparation

Phosphate in the sample will cause the Binding Buffer to precipitate and low IgM purification will result. To remove phosphate ions from ascites fluid, perform a buffer exchange into 20 mM Tris, 1.25 M sodium chloride; pH 7.4. Gel filtration (e.g., D-Salt™ Dextran Desalting Columns, Product No. 43233) or dialysis using a Slide-A-Lyzer® Dialysis Cassette (e.g., Product No. 66382) can be used for buffer exchange. Dilute the dialyzed ascites fluid 1:1 with Binding Buffer.

Warranty: Pierce products are warranted to meet stated product specifications and to conform to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sale for products used, handled and stored according to Pierce instructions. Pierce’s sole liability for the product is limited to replacement of the product or refund of the purchase price. Pierce products are supplied for laboratory or manufacturing applications only. They are not intended for medicinal, diagnostic or therapeutic use. Pierce products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Pierce Biotechnology. Pierce strives for 100% customer satisfaction. If you are not satisfied with the performance of a Pierce product, please contact Pierce or your local distributor.
Gravity-flow Procedure for IgM Purification

This product is designed for optimal isolation and purification of mouse IgM from ascites using the indicated buffers. These instructions may not be valid if other buffers are used. The entire purification procedure will require 8-12 hours to complete. Note that MBP does not bind F(ab’)2 or Fab.

A. Binding

Note: Perform IgM binding at 4°C. Keep the Binding Buffer, sample and immobilized MBP at 4°C.

1. Carefully pack column with the Immobilized MBP according to the packing instructions provided with the columns. For previously packed columns, open column by removing the top cap first and then the bottom cap. Removing the caps in this order prevents air bubble formation in the column, which will impede column flow. Drain the storage solution.

2. Add four gel-bed volumes of Binding Buffer to the column and allow the solution to drain through. An extender (funnel) placed on the top of the column will allow application of the Binding Buffer in larger amounts.

3. Add the cold (4°C) diluted ascites sample to the column and allow it to completely enter the gel.

4. Add 2 ml of Binding Buffer per 5 ml of gel bed to the column and incubate at 4°C for 30 minutes.

5. Wash column with nine gel-bed volumes of the Binding Buffer to remove non-bound protein. Monitor the wash by collecting fractions and measuring their absorbance at 280 nm. Non-bound proteins are removed when the absorbance reaches baseline (i.e., absorbance of the Binding Buffer).

Note: To increase total yield of purified IgM, pool and concentrate flow-through fractions having an absorbance of ≥0.1. This sample may then be reapplied to the Immobilized MBP column.

B. Elution

Note: Perform elution procedure at room temperature.

1. Equilibrate the Elution Buffer and the MBP column to room temperature.

2. Add 3 ml of Elution Buffer to the column for each 5 ml of gel. Allow the Elution Buffer to completely enter the gel. Cap the bottom of the column and incubate upright at room temperature for at least 1 hour.

Note: If desired, the incubation may be extended to overnight. Place the top cap loosely on the column to protect from dust contamination.

3. Remove bottom cap and collect eluate. Collect additional fractions by adding more Elution Buffer. Monitor IgM elution by measuring the absorbance of each fraction at 280 nm. Pool fractions with absorbance measurements that are ≥0.02.

Note: Using a 1 cm cuvette, an absorbance value of 1.18 equals an IgM concentration of 1 mg/ml.

4. Dialyze, desalt or concentrate the IgM fractions with a suitable physiological buffer.

Note: IgM is susceptible to aggregation from multiple freeze-thaw cycles. Store IgM in single-use aliquots of 1-10 mg/ml at -20°C in 50% glycerol in a physiological pH buffer with a buffer salt concentration of 100-200 mM.

5. Wash the column with two gel-bed volumes of 0.02% sodium azide for storage. Cap the bottom and add an additional 1.5 ml of 0.02% sodium azide to the column and then cap the top. Store the column upright at 4°C.

Information Available from the Web

Please visit the Pierce web site for additional information relating to this product including the following items:

- Tech Tip: Protein Stability and Storage
- Tech Tip Protocol: Remove Air Bubbles from Columns
- Tech Tip Protocol: Degas Solutions for use in Affinity Columns
- Tech Tip Protocol: Batch and Spin Cup Methods for Affinity Purification of Proteins
Related Pierce Products

21016 ImmunoPure® IgM Binding Buffer
21017 ImmunoPure® IgM Elution Buffer
53123 UltraLink® Immobilized Mannan Binding Protein, 5 ml settled gel
66382 Slide-A-Lyzer® Dialysis Cassette Kit, 10 dialysis cassettes, each appropriate for 0.5-3.0 ml samples
66526 Slide-A-Lyzer® Concentrating Solution, 10 x 15 ml
23225 BCA™ Protein Assay
69700 Handee™ Spin Cup Columns
29920 Disposable Polystyrene Columns, 0.5-2.0 ml, 100/pkg
29922 Disposable Polypropylene Columns, 1.0-5.0 ml, 100/pkg
29924 Disposable Polypropylene Columns, 2.0-10.0 ml, 100/pkg
29925 Disposable Column Trial Pack, 6 columns (two of each size) plus accessories

References


BCA™ Technology is protected by U.S. Patent # 4,839,295.

The most current versions of all product instructions are available at www.piercenet.com. For a faxed copy, contact customer service (in the USA call 800-874-3723) or your local distributor.