INSTRUCTIONS

Immobilized Mannan Binding Protein

22212

0437.4

Number	Description
22212	**Immobilized Mannan Binding Protein,** 10mL settled gel Support: 4% beaded agarose Capacity: At least 1.5mg of mouse IgM with > 90% purity isolated from a single pass of 0.5mL ascites diluted with 0.5mL of IgG Binding Buffer using a 5mL packed column Supplied: 10mL of gel in 10mL of IgM Binding Buffer (20mL total volume) **Storage:** Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific 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 31kDa. MBP covalently attached to an agarose support produces an excellent tool for affinity purification of IgM.

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: 10mM Tris, 1.25M NaCl, 20mM $CaCl_2$; pH 7.4
- Elution Buffer: 10mM Tris, 1.25M NaCl, 2mM EDTA; pH 7.4 or IgM Elution Buffer (Product No. 21017)
- Disposable column such as the Disposable Polypropylene Columns for 1.0-5.0mL 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., 0.5-2.0mL, 1.0-5.0mL and 2.0-10.0mL gel-bed volumes)

  Note: For spin-column formats, use Thermo Scientific Pierce 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 20mM Tris, 1.25M sodium chloride; pH 7.4. Gel filtration (e.g., Dextran Desalting Columns, Product No. 43233) or dialysis using a Thermo Scientific 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.

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 2mL of Binding Buffer per 5mL 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 280nm. 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 3mL of Elution Buffer to the column for each 5mL 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 280nm. Pool fractions with absorbance measurements that are ≥ 0.02.

   Note: Using a 1cm cuvette, an absorbance value of 1.18 equals an IgM concentration of 1mg/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 1-10mg/mL at -20°C in 50% glycerol in a physiological pH buffer with a buffer salt concentration of 100-200mM.
5. Wash the column with two gel-bed volumes of 0.02% sodium azide for storage. Cap the bottom and add an additional 1.5mL of 0.02% sodium azide to the column and then cap the top. Store the column upright at 4°C.

Additonal Information Available on Our Website

- Tech Tip #43: Protein stability and storage
- Tech Tip #7: Remove air bubbles from columns to restore flow rate
- Tech Tip #29: Degas buffers for use in affinity and gel filtration columns
- Tech Tip #4: Batch and spin cup methods for affinity purification of proteins

Related Thermo Scientific Products

44897	**IgM Purification Kit**
21017	**IgM Elution Buffer,** 500mL
53123	**UltraLink™ Immobilized Mannan Binding Protein**
66382	**Slide-A-Lyzer™ Dialysis Cassette Kit, 10K MWCO, 3mL**
66528	**Slide-A-Lyzer Concentrating Solution**, 200mL
23225	**BCA Protein Assay Kit**
69700	**Pierce Spin Cups – Paper Filter,** 50/pkg

General References

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