INSTRUCTIONS

IgM Purification Kit

44897

0335.4

Number	Description
44897	**IgM Purification Kit**

Kit Contents:

Immobilized MBP Column, 5mL

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 IgM Binding Buffer

Supplied: Prepacked 5mL column in IgM Binding Buffer.

MBP Column Preparation Buffer, 50mL, contains Tris, sodium chloride, sodium azide and EDTA; pH 7.4

IgM Binding Buffer, 800mL, contains Tris, sodium chloride, calcium chloride and sodium azide; pH 7.4

IgM Elution Buffer, 500mL, contains Tris, sodium chloride, sodium azide and EDTA; pH 7.4

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

Introduction

The Thermo Scientific IgM Purification Kit uses immobilized Mannan Binding Protein combined with an optimized buffer system to enable 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.

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.

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. Column Preparation

1. Open the MBP 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. Wash the column with 5mL of the MBP Column Preparation Buffer at room temperature.

B. Binding

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

1. Add 20mL of IgM 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.
2. Add the 1mL of the cold (4°C) diluted ascites sample to the column and allow it to completely enter the gel.
3. Add 0.5mL of Binding Buffer to the column, cap column and incubate at 4°C for 30 minutes.
4. Wash column with 42mL of Binding Buffer to remove non-bound protein. Monitor the wash by collecting 3mL 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.

C. Elution

Note: Perform elution procedure at room temperature.

1. Equilibrate the IgM Elution Buffer and the MBP column to room temperature.
2. Add 3mL of IgM Elution Buffer to the column. 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 (0.5-3mL) 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 10mL of deionized water followed by 10mL of IgM Binding Buffer for storage. Cap the bottom of the column, add an additional 1.5mL of Binding Buffer to the column and then cap the top. Store the column upright at 4°C.

Additional 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

21016	**IgM Binding Buffer**
21017	**IgM Elution Buffer**
22212	**Immobilized Mannan Binding Protein,** 10 ml settled gel
53123	**UltraLink® Immobilized Mannan Binding Protein**, 5 ml settled gel
66382	**Slide-A-Lyzer® Dialysis Cassette Kit**, **10K MWCO, 3mL**
66528	**Slide-A-Lyzer® Concentrating Solution**, 200mL
23225	**BCA™ Protein Assay Kit**

General References

Abdullah, M., *et al.* (1985). The application of DNA-cellulose chromatography in the isolation of immunoglobulin M and complement component C4b-binding protein from human serum. *J Chrom* **347:**129-36.

Cambier, J. and Butler, J. (1974). A rapid method for the purification of immunoglobulin M (IgM) from the sera of certain mammalian species. *Prep Biochem* **4(1):**31-46.

Clezardin, P., *et al.* (1986). Tandem purification of IgM monoclonal antibodies from mouse ascites fluids by anion-exchange and gel fast protein liquid chromatography. *J Chromatogr* **354:**425-33.

Coppola, G., *et al.* (1989). High-performance liquid chromatography of amino acids, peptides and proteins: XCIII. Comparison of methods for purification of mouse monoclonal immunoglobulin M autoantibodies. *J Chromatogr* **476:**269-90.

Meng, Y.G. and Trawinski, J. (1988). Identification of mouse J chain in human IgM produced by an electrofusion-derived mouse-human heterohybridoma. *J Immunol* **141:**2684-7.

Nevins, J.R., *et al.* (1992). Affinity chromatographic purification of immunoglobulin M antibodies utilizing immobilized mannan binding protein. *J Chrom* **597:**247-56.

Ohta, M., *et al.* (1990). The mechanism of carbohydrate-mediated complement activation by the serum mannan-binding protein. *J Biol Chem* **265:**1980-4.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.