

# **IgM Purification Kit**

(Cat# C8056-M; ready-to-use solution; store at 4 °C)

## Introduction

IgM antibody is prominent in early immune responses to most antigens and predominates in certain antibody responses such as 'natural' blood group antibodies. IgM is the major immunoglobulin expressed on the surface of B cells. IgM normally constitutes about 10% of serum immunoglobulins.

ABSbio<sup>™</sup> ready-to-use IgM Purification Kit is formulated for rapid, purification of high-quality IgM from plasma, serum, ascites fluid and tissue culture media, with no binding to other immunoglobulins. The supplied buffers system provide for maximum immunoglobulin binding and elution efficiency with the IgM affinity matrix resin column. IgM affinity matrix exhibits high affinity binding to the Fc part of IgM. The high selectivity and yields obtained using IgM Affinity Matrix enable a robust, fast, and efficient purification process in one step from many species (including human, mouse and rat), and the purified IgM can be used for antibody label, immunoprecipitation assays and immunotherapy.

## Kit Components

IgM Affinity Matrix Column:	1 mL	Binding Buffer:	200 mL
Neutralization Buffer:	10 mL	Elution Buffer:	100 mL

Storage and Handling: Store all of the components at 4 ℃. Shelf Life: 12 months after receipt.

### **Specifications**

 Matrix: Cross-linked poly(styrene-divinylbenzene)

 Bead size range: 50 um

 Ligand: IgM affinity ligand, free of animal components

 Binding capacity: ~ 6 mg/mL (IgM from serum)

 Reusable: stable ligand and resins can be regenerated and reused at least 10 times without significant decline in binding capacity



SDS-PAGE of purified Human IgM

# Applications

Purification of monoclonal and polyclonal antibodies; Immunoglobulin Affinity Purification, Immunoprecipitation



#### Protocol

#### **Preparation of Antibody Samples**

Average IgM concentration in human serum is about 1.5 mg/mL. To ensure proper ionic strength and pH for optimal binding, mix ascites fluid, serum, or tissue culture supernatant sample with Binding Buffer at a ratio of 1:3. Centrifuge samples at 10,000 x g for 5 minutes to remove any insoluble material and use only the clear supernatant for antibody purification.

1. The resin can be used at 2-25 °C. Pour about 1 mL IgM affinity matrix into the column. Remove the bottom cap to allow the storage solution to drain through the column.

2. Slowly add 5-10 mL of Binding Buffer to the top of the resin. Allow the column to drain until liquid level drops to resin level.

3. Add a clarified, diluted antibody sample (5-10 mL) to the column and allow it to slowly pass through the immobilized ligand (Maximum column capacity per purification is about 6-12 mg lgM).

4. Add 10 mL of Binding Buffer and allow it to pass through the column to wash away nonbound components (or until you see a stable baseline).

5. Add 4 mL of Elution Buffer and collect 1 mL elution fractions. Immediately add 100  $\mu$ L of Neutralization Buffer to each of the 1 mL elution fractions.

6. Measuring OD 280nm or Protein assay to identify and combine elution fractions that contain the purified antibody. (Optional) Alternatively, use a desalting column or dialysis to exchange the purified antibody into a more suitable buffer for long-term storage.

7. Regenerate the column by washing with 10 mL of Elution Buffer and followed by 5 mL of 20% Ethanol. When approximately 1 mL of solution remains above the resin, cap the column and store upright at 4°C. Columns may be regenerated a minimal of 9 times without significant loss of binding capacity.

#### **Related Products:**

Protein A Agarose (#C8054) Antibody Purification Kit (#C8056-A) ELISA Washing Buffer(20x, 25mL; #C8003) Protein Loading Buffer(6x ; #C8005) Phosphate Buffered Saline (10x PBS, #C8007) Protein G Agarose (#C8055) Antibody Purification Kit (#C8056-G) Antibody Dilution Buffer(#C8004) SDS-PAGE Running Buffer(10x ; #C8006) Phosphate Buffered Saline with Tween 20(10x PBS-T; #C8008)