Protein A IgG Binding Buffer

rev. 9/13

(Store at 4 ºC)

Cat. No.
6524-1000 Protein A IgG Binding Buffer, 1 L, pH 8.0; contains EDTA as preservative
6524-3750 Protein A IgG Binding Buffer, 3.75 L, pH 8.0; contains EDTA as preservative

I. Introduction:

BioVision's ready-to-use Protein A IgG Binding Buffer is optimized to provide the highest IgG binding efficiency when used with Hi-Bind™ Protein A-Agarose (Cat. # 6520), Protein A-Agarose (Cat. # 6526) & Protein A-Sepharose (Cat. # 6501, 6508) or other protein A resins.

II. Applications:

- Purification of monoclonal and polyclonal antibodies from culture media, serum, ascites fluid or hybridoma supernatants.

III. Content:

Protein A IgG Binding Buffer.

IV. Storage and Handling:

Store the buffer at 4°C. Read the entire protocol before performing the experiment.

V. User Supplied Reagents or Equipment:

- Immobilized Protein A resin (Cat. # 6501, 6508, 6520, 6526), gravity-flow column, IgG Elution buffer (Cat. # 6525) & 1.5 M Tris-HCl pH-8.8 (Cat. # 1105).

VI. Procedure For Purifying IgG:

1. **Sample preparation:** Centrifuge samples at 5000 x g for 15 min. at 4°C. Dilute supernatant at least 1:1 with Protein A IgG Binding Buffer. Make sure the ionic strength and pH are maintained for optimal binding.
2. **Column:** Equilibrate the Buffers & column to room temperature. Carefully pack the column avoiding air bubbles. Equilibrate the column with 5X resin bed volume of Protein A IgG Binding Buffer & allow the Buffer to drain through the column. Do not let the resin bed dry.
3. **Loading:** Add diluted samples to equilibrated column and allow it to flow completely into the resin.

   Note: For max. yield, reapply the flow-through to the column & collect sample. Repeat 1-5 times.
4. **Washing:** Wash the column with at least 5-10 resin-bed volumes of Protein A IgG Binding Buffer.
5. **Elution:** Elute IgG with IgG Elution buffer and collect fractions in micro centrifuge tubes containing neutralization buffer (150 µl of 1.5 M Tris-HCl (pH-8.8) per ml of eluate). Measure the protein concentration by measuring absorbance at 280 nm and combine the fractions with highest absorbance. 1 OD_{280} = 0.73 mg/ml IgG. Use the purified antibodies directly for WB, SDS-PAGE or dialyze for specific application. Purified IgG should be stored at -20°C.

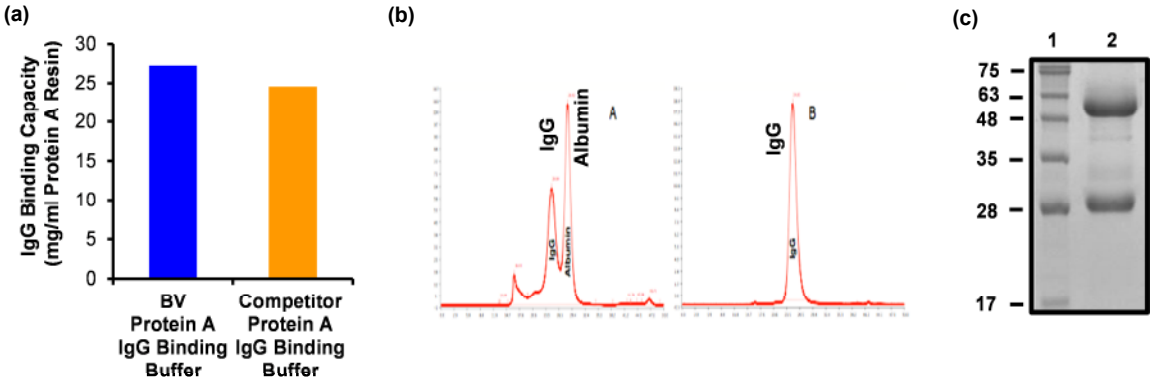

Figure: Purification of IgG using Protein A IgG Binding Buffer – **(a)** BioVision's Protein A IgG Binding Buffer was compared with Protein A IgG Binding Buffer from a competitor. 10 ml rabbit serum in the Binding Buffer was loaded on 2.1 ml Protein A Sepharose & IgG was purified according to the above mentioned protocol. **(b)** SEC analyses of IgG purified from rabbit serum with protein A IgG Binding Buffer. 1 ml rabbit serum in Protein A IgG Binding Buffer was loaded on 1 ml Protein A Sepharose & IgG was purified according to the above mentioned protocol. 200 µl of starting sample (A) (serum) and purified IgG (B) were analyzed on Superdex 200 HR 10/30 column at 0.5 ml/min in 50 mM Tris, 0.25 M NaCl pH 7.5. **(c)** SDS-PAGE (10%) of purified IgG under reduced conditions. Lane 1: Marker, Lane 2: IgG fraction (5 µg).

VI. RELATED PRODUCTS:

Hi-Bind™ Protein A-Agarose (6520)
Protein A-Sepharose (6501, 6508)
Protein A Antibody (5500)
Protein A-Agarose (6526)
Protein A (6500, 6500B)
Protein A-Magnetic Beads (6507)

FOR RESEARCH USE ONLY! Not to be used on humans.