Protein A IgG Purification Buffer Kit

rev. 8/13

(Store at 4ºC)

Cat. No.
K6529-3 Protein A IgG Purification Buffer Kit, contains Protein A IgG Binding Buffer, IgG Elution Buffer & Neutralization Buffer

I. Introduction:

Biovision's ready-to-use Protein A IgG Purification Buffer Kit is optimized to provide the highest IgG yield and purity 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. Kit contents:

Components	K6529-3	Cap Code	Part Number
Protein A IgG Binding Buffer	200 ml	NM	K6529-3-1
IgG Elution Buffer	100 ml	NM	K6529-3-2
Neutralization Buffer	20 ml	NM	K6529-3-3

IV. Storage and Handling:

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

IV. User Supplied Reagents or Equipment:

- Immobilized Protein A resin (Cat. # 6501, 6508, 6520, 6526) or other protein A resins & gravity-flow column.

V. 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 maximum 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 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.

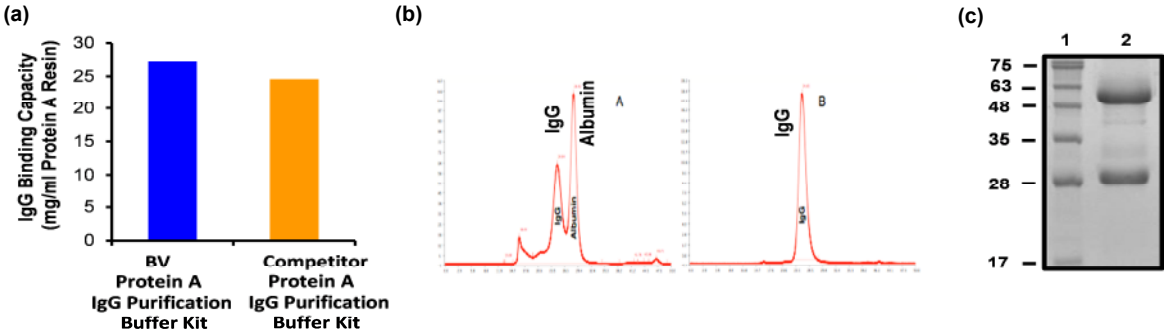

Figure: Purification of IgG using Protein A IgG Purification Buffer Kit – **(a)** BioVision's Protein A IgG Purification Buffer Kit was compared with Protein A IgG Purification Buffer Kit from a competitor. 10 ml rabbit serum in the Binding Buffer was loaded on 2.1 ml Protein A Sepharose & IgG was purified according to the above mentioned protocol. **(b)** SEC analyses of IgG purified from rabbit serum with protein A IgG Purification Buffer Kit. 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.