FOR RESEARCH USE ONLY!

Protein G-Sepharose

rev. 09/16

Store at 4°C. Do not freeze.

Cat. No.	
6511-1	**Protein G-Sepharose, 1 ml settled resin**
6511-5	**Protein G-Sepharose, 5 ml settled resin**
6511-25	**Protein G-Sepharose, 25 ml settled resin**
6511-100	**Protein G-Sepharose, 100 ml settled resin**
6511-1000	**Protein G-Sepharose, 1 L settled resin**

Support: 6% cross-linked Sepharose beads supplied as 50% slurry (e.g., 1 ml of settled resin is equivalent to 2 ml of 50% slurry) in 20% Ethanol/H_2O.

Binding Capacity: >20 mg human or rabbit IgG/ml of settled resin.

Flow Rate Tested*: 0.85 cm/min.
*Test condition: Linear flow rate determined in 2 ml column with internal diameter of 1.5 cm.

Introduction:
Protein G is a cell wall protein produced by group *G streptococcus*. Like protein A, this bacteria-derived protein binds with high affinity & specificity to the Fc portion of most mammalian immunoglobulins. Therefore, Protein G has been widely used for IgG purification. BioVision's Protein G (Cat. # 6510) is a genetically engineered protein containing three Ig-binding regions of native Protein G. The cell wall binding region, albumin binding region and other non-specific regions have been eliminated from the recombinant Protein G to ensure the maximum specific IgG binding. The coupling technique is optimized to give a higher binding capacity for IgG & minimum leaching of recombinant Protein G. In addition, Protein G-Sepharose beads display high chemical & physical stability as well as high flow rate, hydrophilicity & high gel strength. It can be used for IgG purification and immunoprecipitation.

Applications:
- Purification of monoclonal and polyclonal antibodies from culture media, serum, ascites fluid or hybridoma supernatants.
- Isolation of antibody/antigen complexes in immunoprecipitation experiments, since only the Fc region is involved in antibody binding and the Fab region is available for binding antigen.

User Supplied Reagents or Equipment:
- Binding Buffer: PBS/TBS/0.15 M sodium chloride in 50 mM sodium borate, pH 8.0
- Elution Buffer: 0.1 M citric acid, pH 2.75
- Neutralization Buffer: 1 M Tris-HCl, pH-9

Protocol example (Antibody Purification):
1. Carefully pack the column avoiding air bubbles.
2. Equilibrate the column with 5 resin bed volume of Binding Buffer & allow the buffer to drain through the column. Do not let the resin bed dry.
3. Dilute serum sample with Binding Buffer (1:1 ratio).
4. Mix well the diluted serum sample. Make sure there are no bubbles in the sample solution.
5. Apply the diluted sample onto the column. Do not let the resin bed dry.
6. Collect the flow-through.
7. Reapply the flow-through to the column & collect the sample. Repeat 4 times.
8. Wash the column 4 - 5 times with 5 volume of Binding Buffer containing 0.5 M NaCl.
9. Wash the column 4 - 5 times with Binding Buffer.
10. Elute antibodies with Elution Buffer ~3-5 resin bed volume. Collect fractions using micro centrifuge tube containing neutralization buffer (100 µl of 1 M Tris, pH 9.0 per ml of eluate).
11. Assay protein concentration by measuring the absorbance at 280 nm and combine the fractions with highest absorbance. 1 OD_{280} = 0.73 mg/ml IgG.
12. To regenerate/store column:
    a. Wash with 5 volumes of Elution Buffer.
    b. Wash with 5 volumes of distilled water.
    c. Store column in 20 % Ethanol/H_2O at 4°C.

Note: Columns may be regenerated 8-10 times without significant loss of binding capacity.

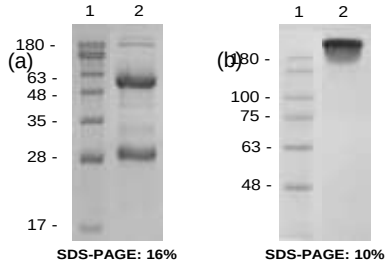

Figure: SDS-PAGE of purified IgG under reduced (a) and non-reduced conditions (b). Lane 1: Marker; Lane 2: IgG fraction (5 µg) purified from human serum using BioVision Protein G Sepharose.

APPENDIX: Protein G affinity for immunoglobulins

Species	Ig	Binding Strength
Human	Total IgG	++++
Human	IgG1	++++
Human	IgG2	++++
Human	IgG3	++++
Human	IgG4	++++
Mouse	Total IgG	++++
Mouse	IgG1	++
Mouse	IgG2a	++++
Mouse	IgG2b	++++
Mouse	IgG3	++++
Rat	Total IgG	++
Rat	IgG1	++
Rat	IgG2a	++++
Rat	IgG2b	+
Rat	IgG2c	++++
Rabbit	Total IgG	++++
Pig	Total IgG	+
Horse	Total IgG	++++
Guinea Pig	Total IgG	+
Cow	Total IgG	++++
Chicken	Total IgG	-
Goat	Total IgG	++++
Dog	Total IgG	+
Cat	Total IgG	+
Sheep	Total IgG	++++

Legend: ++++: Strong Binding
++: Medium Binding
+: Weak Binding
-: No Binding

RELATED PRODUCTS:

Hi-Bind™ Protein A-Agarose (6520)
Protein A-Sepharose (6501)
Protein A-Magnetic Beads (6507)
Protein A (6500, 6500B)
IgG Elution Buffer (6525)
Hi-Bind™ Protein G-Agarose (6513)
Protein G-Sepharose Column (6518)
Protein G (6510)
Protein G Coated 96-well Plate (6522)
Protein L-Sepharose (6531)
Protein L Magnetic Beads (6537)
Protein L (6530)
Protein A/G-Sepharose Column (6528)
Protein A/G (6502)
Protein A/G/L-Sepharose Column (6548)
Protein A/G/L (6540)
Protein A-Agarose (6526)
Protein A-Sepharose Column (6508)
Protein A Antibody (5500)
Protein A IgG Binding Buffer (6524)
Protein A IgG Purification Buffer Kit (6529)
Protein G-Sepharose (6511)
Protein G-Magnetic Beads (6517)
Protein G Antibody (5510)
Protein G-Biotin
Protein L-Sepharose Column (6538)
Protein L Antibody (5530)
Protein A/G-Sepharose (6503)
Protein A/G Magnetic Beads (6527)
Protein A/G/L-Sepharose (6541)
Protein A/G/L Magnetic Beads (6547)

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