# Protein A-Sepharose Column

**CATALOG #:**

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|---|---|
| 6508-1 | 1 ml |
| 6508-5 | 5 ml |

**LOT #:** _____

**PREPARATION:** Protein A Sepharose is prepared by covalently coupling recombinant Protein A to 6 % cross-linked sepharose beads. The coupling technique is optimized to give a high binding capacity for IgG. The capacity of IgG binding could be up to 25 mg of human IgG per ml of wet bead.

**CONTENTS:** Ready-to-use pre-packed columns of 1 ml or 5 ml bead volume in 20 % ethanol/water; > 5 mg Protein A/ml Sepharose beads.

**FEATURES:** High binding capacity (10 - 20 mg/ml of bead);High flow rate; Low falling off of recombinant Protein A.

**APPLICATIONS:** Purification of monoclonal and polyclonal antibodies. Protein A binds to most human and mouse IgG subclasses (e.g., human IgG1, IgG2, IgG4; mouse IgG1, IgG2a, IgG2b, IgG3). It also binds to total IgG from cow, guinea pig, hamster, horse, pig, and rabbit. Protein A has little affinity to chicken, goat, rat and sheep.

**STORAGE:** Store at 4$^{o}$C. Do not freeze. Stable, as supplied, for at least 1 year.

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

**PROCEDURE EXAMPLE:**

1. Equilibrate the column to room temperature.
2. Remove the upper (first) then lower cap and allow the preservative to drain by gravity flow.
3. Equilibrate the column with 5 – 10 bed volumes of degassed Binding Buffer.
4. Add sample in Binding Buffer and recycle through column 15 - 20 times.
5. Wash with 4 – 5 column volumes of Binding Buffer containing 0.5 M NaCl
6. Wash with at least 2 column volumes of Binding Buffer and ensure the effluent reaches the same Absorbance (280 nm) as the Binding Buffer.
7. Drain the column to the top frit. Elute with one bed volume of Elution Buffer buffer pH 3.0. Neutralize with 100 µl of 1M Tris, pH 9.0 per ml of eluate. It is recommended to elute with another 1 - 2 ml of elution buffer and collect 100 µl fractions (test for absorbance at 280 nm).
8. Alternatively, one could add 6 ml Elution Buffer and collect 1 ml fractions into 1.5 ml tubes containing 100 µl of 1 M Tris, pH 9.0.
9. Combine fractions with highest absorbance (Remember to blank the spectrophotometer with a solution containing 100 µl 1 M Tris, pH 9.0 per ml of Elution Buffer).

   Concentration of IgG (mg/ml) = (A280/1.38)
10. Regenerate the column by:
    a. Washing with ~ 5 volumes of Elution Buffer.
    b. Equilibrate with 5 volumes of Binding Buffer containing 20 % ethanol in water.
    c. Store upright at 4 °C.

**BUFFER EXAMPLES:**

(1) **Binding Buffers:** 25 mM sodium phosphate pH 7.2, 50 mM Tris, pH 7.2 or PBS, pH 7.2

(2) **Elution Buffers:** 0.1 M glycine, pH 3.0 or 0.1 M citric acid, pH 2.75

**RELATED PRODUCTS:**

| | |
|---|---|
| Recombinant Protein G- Sepharose Column | Protein G Polyclonal Antibody |
| Recombinant Protein L- Sepharose Column | Protein A Polyclonal Antibody |
| Recombinant Protein A/G- Sepharose Column | Protein L Polyclonal Antibody |
| Recombinant Protein A/G/L- Sepharose Column | Protein G Magentic Beads |
| Recombinant Protein A Sepharose Beads | Protein A Magnetic Beads |
| Recombinant Protein G Sepharose Beads | Protein L Magnetic Beads |
| Recombinant Protein L Sepharose Beads | Protein A/G Magentic Beads |
| Recombinant Protein A/G Sepharose Beads | Protein A/G/L Magnetic Beads |
| Recombinant Protein A/G/L Sepharose Beads | |

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