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ImmunO™

Recombinant Protein A

Catalog #: 98705

Lot #: 81711

Description: Recombinant protein A binds immunoglobulins (primarily IgG) at the Fc region with high affinity. In some cases, IgM and IgA will also bind. This property provides a rapid, simple and economical method for purification and analysis of a broad-spectrum of antibodies. Fractionation of IgG subclasses can also be performed using recombinant protein A.

Because of its high affinity for immunoglobulins of many species and its low level of nonspecific binding, recombinant protein A is useful for the study of cell surface antigens and receptors, and for detecting antibody-secreting cells.

Recombinant protein A is produced in an *E. coli* strain which contains the gene for mature protein A from *Staphylococcus aureus* Cowan I strain¹. Recombinant protein A contains four IgG binding domains labelled D, A,B,C and repeating C-terminal regions labelled S-1 through S-11. These regions are believed to be involved in binding protein A to the cell wall of *Staphylococcus aureus*. No amino acids from the protein A signal sequence are included in the recombinant construction.

Recombinant protein A is a salt-free, lyophilized powder. Protein is determined using biuret methodology.

Purity: 98% as determined by SDS-PAGE gel electrophoresis and HPLC analysis using a TSK-3000 column run in 0.2M NaH₂PO₄, pH 6.8.

Storage: Store the lyophilized product at 2-8°C. For long-term storage, after reconstitution, aliquot and keep frozen at or below -20°C. Avoid repeated freeze/thaw cycles.

Activity: The specific activity of recombinant protein A is equal to the most active protein A preparations extracted from *Staphylococcus aureus*. The disassociation constants for both *Staph. aureus* derived and recombinant protein A to rabbit IgG are 1x10⁻⁹M at pH 7.4, as measured by a solid phase competition RIA.

Protein A and IgG combine to form a precipitation complex. In an assay containing 0.5 mg/ml protein A and varying concentrations of IgG, recombinant protein A will precipitate 3.5 moles of IgG per mole. Protein A derived from *Staph. aureus* precipitates 1.6 moles IgG per mole in this assay. Protein A from both sources reveal a K_{0.5} of 0.15 for the precipitation reaction with a Hill coefficient of 1.75.

Solid-phase ELISA assays show recombinant protein A to have equal or greater binding capacity than several commercial protein A preparations, when used as a "bridge" between purified rabbit IgG and peroxidase conjugated protein A.

Molecular

Weight: The molecular weight of recombinant protein A is 45,000 as determined by SDS-PAGE. This is similar to the 45,216 molecular weight calculated from the gene sequence.

Reference: 1. Colbert, D. et al., **J. Biological Response Modifiers** 3, 255, 1984.


Approved by: Joseph Dietz, Ph.D.
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Control # R097

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