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TECHNICAL INFORMATION

Catalog Number: 150050, 150051, 150052
Protein A

Source: *Staphylococcus aureus*.

Form: Lyophilized, essentially salt free. Protein A is a monomeric protein lacking cysteine residues.

Description: Protein A is a highly stable surface receptor which is capable of binding the Fc portion of immunoglobulins, especially IgGs, from a large number of species.[1] Each protein A molecule can bind 2 molecules of IgG, allowing the formation of a precipitate.[8]

Protein A may be coupled to a wide variety of reporter molecules including fluorescent dyes, enzyme markers, biotin, colloidal gold and radioactive iodine without affecting the antibody binding site. These conjugates may be used to track immunoglobulins in histochemical, western and ELISA applications.

Alternatively, protein A may be immobilized onto a solid support to facilitate the purification and recovery of either polyclonal or monoclonal immunoglobulins. Immobilized protein A has recently been applied therapeutically to treat a variety of humoral diseases.[9]

Molecular Weight: 42,000

Extinction coefficient: $E^{1\%}_{280}$ = 1.4

pH Stability Range: pH 1.0 - 12.0

Isoelectric point: 4.85 - 5.10

Specificity: Protein A will bind the Fc portion of human IgG subclasses, IgM, IgA and IgE; and mouse IgG1 (weakly), IgG2a and IgG2b. Protein A also binds IgGs from other laboratory and domestic animals, including monkey, rabbit, pig, guinea pig, dog and cat.

Antibody	**Affinity for Protein A[10,11]**
Human IgG_1	++++
Human IgG_2	++++
Human IgG_3	-
Human IgG_4	++++
Rat IgG_1	-
Rat IgG_{2a}	-

Rat IgG_{2b}	-
Rat IgG_{2c}	+
Mouse IgG_1	+
Mouse IgG_{2a}	++++
Mouse IgG_{2b}	+++
Mouse IgG_3	++

Assay

The measurement of Protein A binding activity is based upon its adsorption to IgG agarose.

Reagents

– 50 mM Sodium phosphate pH 6.8 containing 150 mM NaCl (PBS)
– 5 ml column of human IgG-agarose (equilibrated with PBS)
– Protein A sample - dissolve at 30 mg/ml in PBS buffer

Procedure

– Adjust spectrophotometer to read at 280 nm.
– Determine the absorbance at 280 nm of a 1:10 dilution of the Protein A sample.
– Load 6 mg of Protein A onto the column.
– Wash with 10 ml of buffer and collect 10 x 1 ml fractions of the eluant.
– Measure the absorbance of the fractions at 280 nm, blanking against buffer.
– The combined absorbance units of the fractions should be less than 5% of the absorbance units loaded onto the column.

Calculation

S$[(FA_{280})(Fu)] < 0.05\ (SA_{280})(Su)$

where:
FA_{280} = Absorbance of each fraction at 280 nm
Fu = Volume of the fractions (1.0 ml)
SA_{280} = Absorbance of undiluted protein A sample at 280 nm
Su = Volume of Protein A sample applied to the column

Availability:

Catalog Number	Description	Size
150050	Protein A, binding capacity: approx. 6-8 mg of human IgG per mg solid	1 mg 5 mg 20 mg
150051	Protein A, binding capacity approx. 9-11 mg of human IgG per mg solid	1 mg 5 mg 20 mg
150052	Protein A, binding capacity approx. 11-14 mg of human IgG per mg solid	1 mg 5 mg 20 mg

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