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User's Manual and Instructions

rProtein A-Sepharose

Catalog Number: L9241005

Introduction

The chromatographic metrix rProtein A-Sepharose is a high capacity, chemically stable affinity medium designed specially for the purification of monoclonal and polyclonal antibodies at laboratory and process scales. Recombinant Protein A was developed to increase the specificity of the molecule for IgG. This modified protein A is expressed from an E.Coli. system, and a specific prification process with strict quality control was taken to get the recombinant protein A with the purity of more than 98% and devoid of bacterial contaminant found normally in native Protein

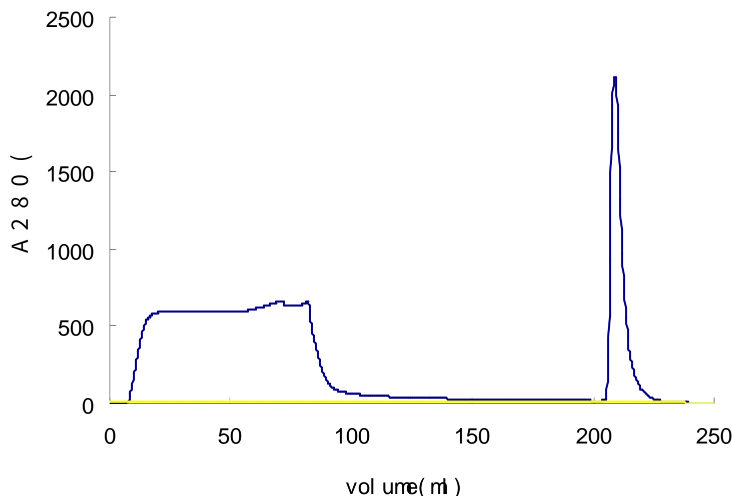
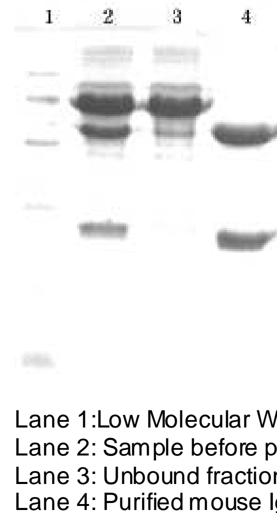


Fig. 1 Separation of mouse IgG_{2a}



Lane 1:Low Molecular Weight marker
Lane 2: Sample before purification
Lane 3: Unbound fraction
Lane 4: Purified mouse IgG_{2a}

Fig. 2 SDS-PAGE of mouse IgG_{2a}

Features

- High IgG binding capacity
- High flow rate
- Low falling off of rProtein A

Applications

- Purification of monoclonal and polyclonal antibodies.

Description

The rProtein A-Sepharose is an affinity chromatographic metrix with recombinant protein A immobilized by the epoxy method to Sepharose 6B Fast Flow. The coupling technique is optimized to give a high binding capacity for IgG. The rProtein A is covalently coupled to the Sepharose with the ligand density of about 6 mg/ml gel. The capacity of IgG binding could be up to 25 mg of human IgG per ml of wet gel.

Characteristics

The supporting matrix Sepharose 6B Fast Flow is a highly cross-linked 6% agarose with high chemical and physical stabilities. It is particularly suitable for process scale applications

where starting material volumes are large and flow rates are high. The resin is compatible with all commonly used buffers and reagents, including high salt and 20% ethanol. The covalent coupling
between the ligand and the metrix is very stable (keep >95% binding capacity over 30 runs). The pH stability range is pH 2-10.

Table 1. rProtein A-Sepharose 6B FF characteristics

Bead structure	Highly cross-linked 6% agarose
Ligand	Modified rProtein
Ligand density	6 mg rProtein A/ml gel
Binding capacity	>25 mg human IgG/ml gel
	>5 mg mouse monoclonal IgG2a/ml gel
	8 mg mouse monoclonal IgG1/ml gel
Mean particle size	50-160 µm
Max. flow rate	200cm/h
Chemical stability	All commonly used buffers
pH stability	
Regular use	3-9
Cleaning	2-10
Temperature stability	
Regular use	+4–37 °C
Storage	+4–8 °C

Operation

rProtein A could bind IgG over a wide pH range, and thus permits the use of a wide variety of buffers, depending on the applications. Elution is often achieved by a decrease in pH. Different
subclasses of IgG elute at different pH values.

Table 2. Affinity of rProtein A for selected classes of monoclonal antibodies.

Antibody	Affinity	Binding pH	Elution pH
Human			
IgG1	++++	6.0-7.0	3.5-4.5
IgG2	++++	6.0-7.0	3.5-4.5
IgG3	+	8.0-9.0	7.0
IgG4	+	7.0-8.0	3.0-6.0
Mouse			
IgG1	+	8.0-9.0	4.5-6.0
IgG2a	++++	7.0-8.0	3.5-5.5
IgG2b	+++	7.0	3.0-4.0
IgG3	+	7.0	3.5-5.5

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Protocol

Buffer preparation
Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.45µm filter before use.
Recommended buffers
Binding buffer: 20 mM Sodium phosphate, pH 7
Elution buffer: 0.1 M Sodium citrate, pH 4-6
Regeneration buffer: 0.1 M Sodium citrate, pH 3
With some antibodies, e.g. mouse IgG1, it might be necessary to add sodium chloride up to 3 M in the binding buffer, to achieve efficient binding.
As a safety measure to preserve the activity of acid labile IgG when using very acidic elution conditions, we recommend adding 60-200 µl of 1 M Tris-HCl, pH 9.0 per ml of eluted fraction, so that the final pH will be approximately neutral.

Sample preparation
The sample should be adjusted to the composition of the binding buffer. This can be done by either diluting the sample with binding buffer or by buffer exchange. The sample should be filtered through a 0.45 µm filter or centrifuged before it is applied to the column.

Purification
a) Wash out the ethanol preservative with at least 5 column volumes of distilled water.
b) Equilibrate the column with 5-10 column volumes of binding buffer.
c) Apply the sample, using a syringe fitted to the luer adaptor or by pumping it onto the column.
d) Wash with 5-10 column volumes of binding buffer or until no material appears in the effluent.
e) Elute with elution buffer. 2–5 column volumes.
f) Regenerate the column with 5 column volumes of regeneration buffer.
g) Wash with 5-10 column volumes of distilled water and 20% ethanol
h) Store the column at +4–8 °C