PurKine™ Protein A/G Resin 4FF

Cat #: BMR2070 Size: 2 mL/10 mL

	Protein A/G Resin 4FF, crosslinked 4% agarose		
REF	**Cat #:** BMR2070	LOT	**Lot #:** Refer to product label
	Capacity: >10 mg IgG protein/mL		**Bead size:** 45-165 μm
	Tolerance: 0.1 MPa, 1 bar		**Buffer:** PBS containing 20% ethanol
	Storage: Stable for 12 months at 4℃ from date of shipment		

Assay Principle

Protein A and G are popular choices for antibody purification, because they are both stable and target selective. IgG class antibodies from multiple species bind to protein A and/or G, allowing antibody to be captured on protein-agarose microspheres. Protein A and G bind IgG subtypes with varying affinities, determined by species and the properties of the heavy chain. PurKine™ Protein A/G Resin 4FF provides a simple, rapid, and efficient purification of antibodies and Immunoprecipitation.

Reagent Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.22 or 0.45 μm filter before use.

Binding/Wash Buffer: 0.15 M NaCl, 20 mM Na_2HPO_4, pH 7.0

Elution Buffer: 0.1 M Glycine, pH 3.0

Neutralization Buffer: 1 M Tris-HCl, pH 8.5

Sample Preparation

To insure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascitic fluid or cell culture supernatant at least 1:1 with Binding/Wash Buffer. Alternatively, the sample may be dialyzed overnight against Binding/Wash Buffer. It is recommended filtering the sample solution by passing them through a 0.22 μm or 0.45 μm filter before use.

Procedure for Sample Purification

1. Pack column with an appropriate amount of Protein A/G Resin 4FF. Allow storage buffer to drain from resin by gravity flow.
2. Add 2 resin-bed volume binding buffer to the column. Equilibrate the column, and drain away the binding buffer. Repeat this step for three times.
3. Add the prepared sample (Prepare sample by mixing protein extract with equal binding Buffer) to the column, collect the effluent liquid which can be analyzed by SDS-PAGE.

Note: For maximal binding, the sample can be incubated for 30 min at room temperature or 4℃. Be careful not to exceed the resin's binding capacity.

4. Add 2 resin-bed volume wash buffer to the column to remove the non-specific adsorption protein. Collect the wash liquid

which can be analyzed by SDS-PAGE. Repeat this step for six times.

5. Add 5-10 resin-bed volume elution buffer to the column to wash the target protein, or until the absorbance of the effluent at 280 nm is stable. Collect the eluate containing the target immunoglobulin and immediately neutralize to pH 7.4 with 1 M Tris-HCl, pH 8.5 (1/10 volume of total eluate).

6. Examine and identify the fractions containing the target protein. Use UV absorbance, SDS-PAGE, or Western blotting.

Storage of the Column

Use 2 resin-bed volume Binding Buffer and 2 resin-bed volume deionized water to equilibrate the column in turn, repeat twice. Then add 2 resin-bed volume 20% ethanol, repeat once. Add equal volume PBS containing 20% ethanol as storage buffer, store the column in 4℃ to keep bacteria away.

Cleaning-in-Place (CIP)

In general, resins are well suited for reuse at least five times. When precipitation and protein aggregation cause the loss of velocity and combined loads, you need to clean the medium.

To remove the precipitation or denatured protein: Wash the column with 2 column volumes 6 M guanidine hydrochloride solution. Finally wash the column with 5 column volumes PBS (pH 7.4).

To remove the non-specific adsorption protein: Wash the column with 3 column volumes 70% ethanol or 1% Triton X-100. Finally wash the column with 5 column volumes PBS (pH 7.4).

Trouble Shooting

Problem	Cause	Solution
High back pressure	Column is clogged	Cleaning-in-place
	Sample solution contains precipitate	Filtering the sample solution by passing them through a 0.22 μm or 0.45 μm filter
No antibody is detected in any elution fraction	The concentration of antibody of interest is very low	Purify the antibody using the specific antigen coupled to resin
	The antibody is sensitive to low-pH elution buffer	Neutralize the eluted fractions with Neutralization Buffer immed iately after elution
	The IgG subclass does not bind to the resin that you choose	Try other affinity chromatography media to purify the antibody

Recommended Products

Catalog No.	Product Name
KTP2070	PurKine™ Antibody Purification Kit (Protein A/G)
KTP2001	PurKine™ His-Tag Protein Purification Kit (Ni-NTA)
KTP2010	PurKine™ GST-Tag Protein Purification Kit (Glutathione)
KTP2020	PurKine™ MBP-Tag Protein Purification Kit (Dextrin)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.