

Protocol for PurKine™ Protein A Products

Item NO.	Product Name
BMR20500	PurKine™ Protein A Resin
BMC20500	PurKine™ Protein A Packed Column
KTP20500	PurKine™ Antibody Purification Protein A Kit
BMR20504	PurKine™ Protein A Resin 4FF



ATTENTION

For laboratory research use only

Not for clinical or diagnostic use

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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 0.45µm filter before use.

Binding/Wash Buffer: 0.15 M NaCl, 20 mM Na₂HPO₄, 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, ascite 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 0.22µm or 0.45µm filter before use.

Protocol for Sample Purification

1. Fix Column. Move the top and bottom stopper, and let the storage buffer drain away.
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 minutes at room temperature or 4°C. 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.

After-use Storage

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 1xPBS containing 20% ethanol as storage buffer, store the column in 4°C 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 6M guanidine hydrochloride solution. Finally wash the column with 5 column volumes 1XPBS (pH7.4).

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 1XPBS (pH7.4).

Troubleshooting

Problem	Probable cause	Solution
High back pressure	Column is clogged	Cleaning in place.
	Sample solution contains precipitate	Filtering the sample solution by passing them through a 0.22µm 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 immediately after elution.
	The IgG subclass does not bind to the resin that you choose	Try other affinity chromatography media to purify the antibody.

Related PurKine™ products

Item NO.	Product Name
BMR20600	PurKine™ Protein G Resin
BMC20600	PurKine™ Protein G Packed Column
KTP20600	PurKine™ Antibody Purification Protein G Kit
BMR20604	PurKine™ Protein G Resin 4FF
BMR20704	PurKine™ Protein A/G Resin 4FF
KTP20704	PurKine™ Antibody Purification Protein A/G Kit
BMR20800	PurKine™ Protein L Resin
BMC20800	PurKine™ Protein L Packed Column
BMR20804	PurKine™ Protein L Resin 4FF
BMC20804	PurKine™ Protein L Packed Column 4FF

BMR20904
BMC20904

PurKine™ Protein AT Resin 4FF
PurKine™ Protein AT Packed Column 4FF