Protocol for PurKine™ Anti-DDDDK Tag Resin 4FF

Item NO.	Product Name
BMR21504	PurKine™ Anti-DDDDK Tag Resin 4FF

ATTENTION

For laboratory research use only

Not for clinical or diagnostic use

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Reagent Preparation

It is recommended to filter all buffers before use by passing through a 0.22μm or 0.45 μm filter. For most proteins, the following buffer are recommended:

Binding Buffer: 0.15M NaCl, pH 7.0
Wash Buffer: Tris-buffered saline, TBS (50 mM Tris-HCl, 150 mM NaCl, pH 7.4)
Elution Buffer (according to protein characteristics or further usage):
1) Elution under native conditions: TBS solution contains 100-500μg/ml DYKDDDDK peptide
2) Elution under acidic conditions: 0.1 M Glycine HCl, pH 3.5
3) Elution under alkaline conditions: 0.1 M Tris, 0.5 M NaCl, pH 12.0

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 wash liquid, and analyzed the content in each tube respectively.
6. Examine and identify the fractions containing 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)

A column used to purify protein from cell extract usually has buildup of insoluble substances and cell debris, which are non-specifically absorbed onto the matrix support and cannot be completely removed during washing steps. If the column is to be reused, these contaminants should be cleaned from the column. Cleaning-in-Place helps eliminating materials that cannot be removed by regeneration and preventing progressive buildup of contaminants.

1. Wash with 3 column volumes of 0.1 M Tris HCl, 0.5 M NaCl, pH 8.0;
2. Wash with 3 column volumes of 0.1 M sodium acetate, 0.5 M NaCl, pH 4.0;
3. Equilibrate with 3-5 column volumes TBS;
4. For long-term use, it is recommended that the resin is kept in the TBS solution containing 0.02% sodium azide in 2-8°C.

Related PurKine™ products

Item NO.	Product Name
BMR21206	PurKine™ Heparin Resin 6FF
BMR21306	PurKine™ Benzamidine Resin 4FF