

Protocol for PurKine™ MBP Purification Products

Item NO.	Product Name
BMR20206	PurKine™ MBP-Tag Dextrin Resin 6FF
BMC20206	PurKine™ MBP-Tag Dextrin Packed Column 6FF
KTP20206	PurKine™ MBP-Tag Protein Purification Kit (Dextrin)



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

Binding/Wash Buffer: 20mM Tris-HCl, 200mM NaCl, 1mM EDTA, pH7.4

Elution Buffer: 20mM Tris-HCl, 1mM EDTA, 10mM Maltose, pH7.4

Note: 1mM DTT or 10mM β-mercaptoethanol can be included in the binding and elution buffer.

Sample Preparation

The sample should be centrifuged and/or filtered through a 0.22µm or 0.45µm filter before it is applied to the medium to prevent clogging the column. If the sample is too viscous, dilute it with binding buffer to prevent clogging the column. Be careful not to exceed the resin's binding capacity.

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 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, resin may be used at least five times. When 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 and preventing progressive buildup of contaminants.

1. Add 3 column volumes of deionized water
2. Add 3 column volumes of 0.1% SDS or 0.5 M NaOH solution
3. Add 3 column volumes of deionized water. Add equal volume 20% ethanol as storage buffer, store the column in 4°C.

Troubleshooting

Problem	Probable cause	Solution
Back pressure exceeds	Column is clogged	Cleaning-in-place. Increase the centrifugation speed or filtering the sample
No protein is eluted	Expression of target protein in extract is very low	Check expression level of protein by estimating the amount in the extract, flow through, elute fraction and pellet upon centrifugation. Or apply larger sample volume
	Target protein is degraded	Perform purification at 4°C in the presence of protease inhibitors
	Lots of amylase exist in sample or buffer	Add glucose in culture medium to inhibit amylase expression

Related PurKine™ products

Item NO.	Product Name
A02070	Anti-MBP Tag Mouse Monoclonal Antibody (9Y5)
BMR20100	PurKine™ GST-Tag Glutathione Resin
BMC20100	PurKine™ GST-Tag Glutathione Packed Column
KTP20100	PurKine™ GST-Tag Protein Purification Kit (Glutathione)

BMR20104	PurKine™ GST-Tag Glutathione Resin 4FF
BMC20104	PurKine™ GST-Tag Glutathione Packed Column 4FF
BMR20306	PurKine™ Biotin-Tag Streptavidin Resin 6FF
BMC20306	PurKine™ Biotin-Tag Streptavidin Packed Column 6FF
KTP20306	PurKine™ Biotin-Tag Protein Purification Kit (Streptavidin)
BMR20400	PurKine™ Strep II-Tag Strep-Tactin Resin 4FF