Protocol for PurKine™ GST Purification Products

Item NO.	Product Name
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

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: PBS, pH 7.4 (140 mM NaCl, 2.7mM KCl, 10mM Na_2HPO_4, 1.8mM KH_2PO_4, pH 7.4)
Elution Buffer: 50mM Tris-HCl, 20mM GSH, pH 8.0

Note: 1–10 mM DTT can be included in the binding and elution buffer to increase purity. However, this may result in lower yield of target protein.

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. Avoid using protease inhibitors or other additives that contain chelators, such as EDTA, or strong reducing agents, such as DTT or β-mercaptoethanol, which will disrupt the function of the resin. 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.

To removal of precipitated or denatured substances:

Wash with 2 column volumes of 6 M guanidine hydrochloride, immediately followed by 5 column volumes of PBS, pH 7.4.

To removal of hydrophobically bound substances:

Wash with 3~4 column volumes of 70% ethanol or 2 column volumes of 1% Triton™ X-100, immediately followed by 5 column volumes of PBS, pH 7.4.

Troubleshooting

Problem	Probable cause	Solution
The yield of the purified GST fusion protein is low or undetectable	The fusion protein forms inclusion body	Grow bacteria at lower temperature(20-30°C), or reduce final concentration of IPTG to 0.1mM for protein induction, or reduce the induction time; Properly dissolve and refold the inclusion body prior to the purification
	The fusion protein does not contain active GST	Use mild sonication condition or other lysis method, such as lysozyme so that GST is not denatured
	The fusion protein is degraded by protease	Add appropriate protease inhibitors such as PMSF in the lysis solution
Multiple bands observed in the eluted protein	The fusion protein is degraded by protease	Add appropriate protease inhibitors such as PMSF in the lysis solution
	Some host proteins, such as chaperonins, may interact with the fusion protein	Incubate the recombinant protein solution in chaperonin buffer (2mM ATP, 10mM $MgSO_4$, 50mM Tris-HCl) at 37°C for 10 min prior to the purification
	Over-sonication will cause some protein to bind to the fusion	Use milder sonication condition or another lysis method

	protein	

Related PurKine™ products

Item NO.	Product Name
A02030	Anti-GST Tag Mouse Monoclonal Antibody (2A8)
BMR20000	PurKine™ His-Tag Ni-IDA Resin
BMR20010	PurKine™ His-Tag Ni-NTA Resin
BMC20010	PurKine™ His-Tag Ni-NTA Packed Column
KTP20010	PurKine™ His-Tag Protein Purification Kit (Ni-NTA)
BMR20016	PurKine™ His-Tag Ni-NTA Resin 6FF
BMC20016	PurKine™ His-Tag Ni-NTA Packed Column 6FF
BMR20040	PurKine™ His-Tag Cu-IDA Resin
BMR20050	PurKine™ His-Tag Co-NTA Resin
BMR20056	PurKine™ His-Tag Co-NTA Resin 6FF
BMR20036	PurKine™ His-Tag IMAC-NTA Resin 6FF
BMR20206	PurKine™ MBP-Tag Dextrin Resin 6FF
KTP20206	PurKine™ MBP-Tag Protein Purification Kit (Dextrin)
BMR20306	PurKine™ Biotin-Tag Streptavidin Resin 6FF