Protocol for PurKine™ His-Tag Ni-Super Purification Products

Item NO.	Product Name
BMR20020	PurKine™ His-Tag Ni-Super Resin
BMC20020	PurKine™ His-Tag Ni-Super Packed Column
BMR20026	PurKine™ His-Tag Ni-Super Resin 6FF
BMC20026	PurKine™ His-Tag Ni-Super Packed Column 6FF

ATTENTION

For laboratory research use only

Not for clinical or diagnostic use

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Chemical Compatibilities

Reagent	Duration
0.01 M HCl, 0.01M NaOH	One week
10mM EDTA, 1M NaOH, 5mM DTT, 5mM TCEP, 20mM β-ME, 6M guanidine-HCl	24 hours
500mM imidazole, 100mM EDTA	2 hours
30% isopropanol	20 minutes

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.

Lysis Buffer: 20mM sodium phosphate, 0.5M NaCl, pH7.4
Wash Buffer: 20mM sodium phosphate, 0.5M NaCl, up to 5mM imidazole, pH7.4
Elution Buffer: 20mM sodium phosphate, 0.5M NaCl, 250mM imidazole, pH7.4

Note:
1. It is not recommended to include imidazole in the sample and lysis buffer. To minimize host cellular proteins in the eluate, imidazole can be added to a low concentration in wash buffer. However, the concentration of imidazole should be determined empirically. For some target proteins, even a small increase of the imidazole concentration in wash buffer may lead to partial elution.
2. Adding salt (e.g. 0.5 ~ 1.0 M NaCl) to buffers may help minimizing ion-exchange effects.

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. 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 nickel 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 lysis buffer to the column. Equilibrate the column, and drain away the lysis buffer. Repeat this step for three times.
3. Add the prepared sample (Prepare sample by mixing protein extract with equal lysis 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 His-tagged protein. Use UV absorbance, SDS-PAGE, or Western blotting.

After-use Storage

Use 2 resin-bed volume lysis 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.

Regeneration

Ni-Super Resin (6FF) are very strongly bound to the ligand and will remain bound to the ligand even after incubation with 1 M NaOH or 10 mM EDTA. Also after the extreme conditions of incubating with 100 mM EDTA for 2 hours, a lot of the nickel remains bound. Therefore, regeneration after normal use or CIP, even after several normal runs, is not necessary.

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 the column using 2-5 resin-bed 0.1-0.3 M NaOH once.
2. Finally wash the column with 10CV distilled water.

Troubleshooting

Problem	Probable cause	Solution
Back pressure exceeds	Column is clogged	Cleaning-in-place. Increase the centrifugation speed or filtering the sample
	Sample is too viscous	Increase sonication or add DNase I (5 μg/ml with 1mM Mg^{2+}). Incubate on ice for 15min

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
Protein precipitates during purification	Temperature is too low	Perform the purification at room temperature
	Aggregate formation	Add solubilizing agents to the samples and buffers, for example 0.1% Triton X-100, Tween-20 and ≤20% glycerol to maintain protein solubility

Related PurKine™ products

Item NO.	Product Name
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