PurKine™ His-Tag Ni-NTA Resin

Cat #: BMR2001 Size: 5 mL/25 mL

	His-Tag Ni-NTA Resin, crosslinked 4% agarose		
REF	**Cat #:** BMR2001	LOT	**Lot #:** Refer to product label
	Capacity: >40 mg 6×His -tagged protein/mL		**Bead size:** 45-165 μm
	Tolerance: 0.1 MPa, 1 bar		**Buffer:** PBS containing 20% ethanol
	Storage: Stable for 12 months at 4℃ from date of shipment		

Assay Principle

PurKine™ His-Tag Ni-NTA Resin enables effective immobilized metal affinity chromatography (IMAC) purification of polyhistidine-tagged proteins from a soluble protein extract. This resin is composed of nickel-charged nitrilotriacetic acid (NTA) chelate immobilized onto 4% crosslinked agarose. Ni-NTA resins are commonly chosen for His-tagged-protein purification because of the four metal-binding sites on the chelate, which allow for high-binding capacity and low-metal ion leaching. Besides, Ni-NTA resins can withstand severe conditions such as reducing agent, denaturant or coupling agent with a certain concentration, and has wider applicability, more stable ligand and higher selectivity.

Reagent Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter all buffers before use by passing through a 0.22 μm or 0.45 μm filter. For most proteins, the following buffer are recommended:

Lysis Buffer: 50 mM NaH_2PO_4, 300 mM NaCl,10 mM Imidazole, pH 8.0;

Wash Buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 25 mM Imidazole, pH 8.0;

Elution Buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 250 mM Imidazole, pH 8.0;

Note: Sometimes overexpressed proteins are sequestered in inclusion bodies. Inclusion bodies of His -tagged proteins can be solubilized in 8 M urea or 6 M guanidine. We recommend you use the buffer below:

Lysis buffer: 8 M Urea, 100 mM NaH_2PO_4, 100 mM TrisHCl, pH 8.0;

Wash buffer: 8 M Urea, 100 mM NaH_2PO_4, 100 mM TrisHCl, pH 6.3;

Elution buffer: 8 M Urea, 100 mM NaH_2PO_4, 100 mM TrisHCl, pH 4.5 .

Sample Preparation

The sample should be centrifuged and/or filtered through a 0.22 μm or 0.45 μm 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.

Procedure for Sample Purification

1. Pack column with an appropriate amount of Ni-NTA Resin. Allow storage buffer to drain from resin by gravity flow.
2. Add 5 resin-bed volume Lysis Buffer to the column. Equilibrate the column (make the Ni-NTA Resin in the same buffer system as the target protein to protect the protein). Allow buffer to drain from the column.
3. Add the prepared protein extract to the resin. (In order to improve the recovery rate of the target protein, the adding speed was controlled to ensure the full contact between the target protein and Ni^{2+}).
Note: Collect the flow-through which can be analyzed by SDS-PAGE. When problems arise, it is easier to find solutions.
4. Add 10-15 resin-bed volume Wash Buffer to the column to remove the non-specific adsorption protein. Pay attention to collecting the flow-through.
5. Add 5-10 resin-bed volume Elution Buffer to the column to wash the target protein. The collected eluate is the target protein solution.
6. Add 3 resin-bed volume Lysis Buffer and 5 resin-bed volume deionized water to the column in turn to equilibrate the Ni-NTA Resin. Store resin in an equal volume of 20% ethanol at 4-30℃ to prevent the resin from being contaminated by bacteria.
7. The flow-through eluted protein and prepared protein extract can be directly analyzed by SDS-PAGE.

Cleaning-in-Place (CIP)

When the back pressure is too high or obvious contamination appears on the resin during the use of the resin, it needs to be cleaned-in-place (CIP). It is recommended to follow the steps below to remove residual contaminants on the resin, such as precipitated proteins, hydrophobins, and lipoproteins.
To remove strongly bound hydrophobic proteins, lipoproteins and lipids: Wash the column using 5-10 resin-bed volumes of 30% isopropanol contacting for 15-20 min. Or apply 2 resin-bed volumes of acidic or alkaline solution containing detergent (i.e. 0.1 M acetic acid solution contains 0.1-0.5% non-ionic detergent), for 1-2 h. Finally wash the column with 10 resin-bed volumes of distilled water.
To remove the proteins engaged with ionic interaction: Wash the column with 1.5 M NaCl for 10-15 min. Finally wash the column with 10 resin-bed volumes of distilled water.

Ni-NTA Resin Regeneration

In general, The Ni-NTA resin may be used at least five times before it becomes necessary to recharge them with metal ions. When the back pressure is too high or the capacity significantly lower, it needs to strip the metal ions and recharge the resin as the following procedure:
1. Wash resin with 5 resin-bed volumes of deionized water; 2. 100 mM EDTA (pH 8.0), 5 resin-bed volumes; 3. Wash resin with 10 resin-bed volumes of deionized water; 4. Wash resin with 5 resin-bed volumes of 0.5 M NaOH and stay for 10-15 min; 5. Wash resin with 10 resin-bed volumes of deionized water; 6. 100 mM $NiSO_4$, 3-5 resin-bed volumes; 7. Wash resin with 10 resin-bed volumes of deionized water.
After regeneration, the medium can be used immediately or store in 20% ethanol at 4℃.

Recommended Products

Catalog No.	Product Name
KTP2001	PurKine™ His-Tag Protein Purification Kit (Ni-NTA)
ABT2050	Anti-His Tag Mouse Monoclonal Antibody (5C3)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.