

DP6805 Ni DePur Beads 6FF

1. Product Description

Ni DePur Beads 6FF is a novel IMAC resin with strongly chelated Ni ions. Key performance parameters are listed in Table 1. Ni DePur Beads 6FF is mainly used for capture and purification of histidine-tagged proteins secreted into eukaryotic culture supernatants, enabling efficient purification even in the presence of EDTA and DTT. The resin is also suitable for purifying intracellularly expressed proteins from various expression systems, with higher selectivity and better target protein purity. Ni DePur Beads 6FF exhibits excellent buffer compatibility, performing well across a wide range of substrates and salt concentrations. Reagent tolerance is summarized in Table 2.

Table 1. Specifications of Ni DePur Beads 6FF

Item	Description
Matrix	Highly cross-linked 6% agarose beads
Binding Capacity	> 10 mg 6×His-tagged protein/mL resin
Particle Size	45–165 μm
Maximum Pressure	0.3 MPa, 3 bar
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	4–30 °C

Table 2. Chemical Compatibility of Ni DePur Beads 6FF

Reagent	Tolerance Duration
0.01 M HCl, 0.01 M NaOH	1 week
10 mM EDTA, 1 M NaOH, 5 mM DTT, 5 mM TCEP, 20 mM β-mercaptoethanol, 6 M Gu-HCl	24 hours

Reagent	Tolerance Duration
500 mM imidazole, 100 mM EDTA	2 hours
30% isopropanol	20 min

2. Purification Procedure

2.1 Buffer Preparation

Use the recommended buffers below or prepare custom formulations following the core principles: low imidazole for loading, high imidazole for elution; or high pH for loading, low pH for elution. Filter all buffers through a 0.22 µm or 0.45 µm membrane before use. Ni DePur Beads 6FF supports purification of soluble proteins and inclusion bodies with different buffer requirements as follows.

Buffers for Soluble His-Tagged Protein Purification

- **Lysis Buffer:** 20 mM NaH₂PO₄, 500 mM NaCl, pH 8.0
- **Wash Buffer:** 20 mM NaH₂PO₄, 500 mM NaCl, 0–5 mM imidazole, pH 8.0
- **Elution Buffer:** 20 mM NaH₂PO₄, 500 mM NaCl, 250 mM imidazole, pH 8.0

Buffers for Inclusion Body His-Tagged Protein Purification

- **Lysis Buffer:** 20 mM NaH₂PO₄, 500 mM NaCl, 8 M urea, pH 8.0
- **Wash Buffer:** 20 mM NaH₂PO₄, 500 mM NaCl, 8 M urea, 0–5 mM imidazole, pH 8.0
- **Elution Buffer:** 20 mM NaH₂PO₄, 500 mM NaCl, 8 M urea, 250 mM imidazole, pH 8.0

Note: Add low concentrations of imidazole to the Wash Buffer to reduce host protein binding. Add 0.5–1.0 M NaCl to minimize ionic interactions. Low-pH elution increases elution volume and may be used as needed.

2.2 Sample Preparation

2.2.1 Proteins Expressed in Bacteria or Yeast

1. Inoculate a single colony into culture medium and induce expression per the vector instructions.
2. Harvest cells by centrifugation at 7,000 rpm (7,500×g) for 15 min at 4 °C. Discard supernatant. Resuspend the pellet in Lysis Buffer at a 1:10 (w/v) ratio. Add PMSF to 1 mM and lysozyme (0.2–0.4 mg/mL; omit if host contains pLysS/pLysE).

3. For high-density suspensions, add 10 µg/mL RNase A and 5 µg/mL DNase I. Sonicate on ice until the lysate is clear.
4. Clarify the lysate by centrifugation at 10,000 rpm (15,000×g) for 20–30 min at 4 °C. Collect the supernatant for immediate use or storage at -20 °C.

2.2.2 Soluble Proteins Secreted by Eukaryotic Cells (Yeast, Insect, Mammalian)

1. Centrifuge cell culture at 7,000 rpm (7,500×g) for 15 min at 4 °C. Collect the supernatant.
2. If the supernatant contains compatible reagents, load directly without dialysis/concentration.

Note: Filter all samples through a 0.22 µm or 0.45 µm membrane before loading to prevent column clogging.

2.2.3 Inclusion Body Purification (Denaturing Conditions)

1. Harvest cells by centrifugation at 7,000 rpm (7,500×g) for 15 min at 4 °C. Discard supernatant.
2. Resuspend the pellet in Lysis Buffer (without urea) at a 1:10 (w/v) ratio. Sonicate on ice.
3. Centrifuge at 10,000 rpm (15,000×g) for 20–30 min at 4 °C. Discard supernatant. Repeat steps 2–3 once.
4. Resuspend the inclusion body pellet in denaturing Lysis Buffer (with 8 M urea) at a 1:10 (w/v) ratio.
5. Purify the His-tagged protein under denaturing conditions.

2.3 Column Packing

2.3.1 Gravity Column Packing

1. Place a lower frit in a gravity column. Rinse the column and frit with water. Close the outlet.
2. Resuspend Ni DePur Beads 6FF. Add the slurry to the column. Open the outlet to drain storage solution.
3. Rinse the resin with water. Drain completely. Close the outlet.
4. Place a pre-rinsed upper frit, ensuring no gaps with the resin bed.
5. Equilibrate the packed column with equilibration buffer.

2.3.2 Medium-Pressure Column Packing

Ni DePur Beads 6FF is widely used for industrial purification. Calculate resin volume:

$$V = 1.15 \pi r^2 h$$

- V: resin volume (mL)
- $\sqrt[3]{1.15}$: compression factor
- r: column radius (cm)
- h: bed height (cm)

Note: Use twice the calculated slurry volume (resin = 50% of slurry volume).

1. Rinse the column frit and adapter with water. Remove air bubbles. Close the outlet, leaving 1–2 cm water at the bottom.
2. Resuspend the resin. Pour the slurry down a glass rod against the column wall to avoid bubbles.
3. If using a reservoir, fill the column and reservoir with water. Attach the adapter to the slurry surface. Connect to a pump.
4. Open the outlet. Start the pump at low flow, gradually increasing to final velocity. Do not exceed 75% of packing flow rate in subsequent runs. After bed stabilization, flow 3 column volumes of water. Mark bed height.
5. Stop the pump. Close the outlet.
6. Remove the reservoir. Position the adapter at the marked bed height.
7. Lock the adapter. Connect to a chromatography system. Equilibrate.

2.4 Sample Purification

2.4.1 Batch Purification

1. Transfer resin to a tube. Centrifuge at 1,000 rpm for 1 min. Discard supernatant.
2. Wash resin twice with 5 column volumes Lysis Buffer. Centrifuge and discard supernatant.
3. Add sample. Incubate at 4 °C for 2–4 h or 37 °C for 30 min–2 h with agitation.
4. Centrifuge at 1,000 rpm for 1 min. Collect supernatant (flow-through).
5. Wash resin 3–5 times with 5 column volumes Wash Buffer. Centrifuge and discard supernatant.
6. Elute with 3–5 column volumes Elution Buffer. Incubate 10–15 min at room temperature. Collect eluate. Repeat 2–3 times.

2.4.2 Gravity Column Purification

1. Equilibrate the column with 5 column volumes Lysis Buffer. Repeat 2–3 times.

2. Load sample. Allow ≥ 2 min residence time. Collect flow-through. Reload for higher binding.
3. Wash with 10–15 column volumes Wash Buffer. Collect wash fractions.
4. Elute with 5–10 column volumes Elution Buffer. Collect 1 column volume per fraction.

2.4.3 Medium-Pressure Column Purification

1. Fill pump tubing with water. Connect the column to the system. Open the outlet.
2. Rinse storage solution with 3–5 column volumes water.
3. Equilibrate with ≥ 5 column volumes Lysis Buffer.
4. Load sample via pump or loop.
5. Wash with Wash Buffer until UV absorbance stabilizes (≥ 10 –15 column volumes).
6. Elute with Elution Buffer via step or linear gradient.

Post-purification: Rinse with 3 column volumes Lysis Buffer → 5 column volumes water → 2 column volumes 20% ethanol. Store at 2–8 °C.

2.5 SDS-PAGE Analysis

Analyze fractions (flow-through, wash, eluate) and starting material by SDS-PAGE.

3. Cleaning-in-Place (CIP)

Perform CIP for high backpressure or visible contamination.

Remove Strong Hydrophobic Proteins, Lipoproteins, Lipids

- 5–10 column volumes 30% isopropanol (15–20 min contact). Rinse with 10 column volumes water.
- Or 2 column volumes acidic/alkaline detergent solution (e.g., 0.1 M acetic acid + 0.1–0.5% nonionic detergent, 1–2 h contact). Rinse with 5 column volumes 70% ethanol → 10 column volumes water.
- Or 3 column volumes 0.1 M/0.5 M NaOH. Rinse with 10–15 column volumes water.

Remove Ion-Bound Proteins

- 1.5 M NaCl (10–15 min contact). Rinse with 10 column volumes water.
- Rinse with 2 column volumes 20% ethanol. Store at 2–8 °C.

4. Troubleshooting

Problem	Cause	Solution
High backpressure	Clogged resin	Perform CIP (Section 3).
	Particulates in lysate	Filter sample (0.22/0.45 µm) or centrifuge.
Viscous sample	High nucleic acid content	Increase sonication. Add DNase I (5 µg/mL) + 1 mM Mg ²⁺ , 10–15 min on ice.
	Viscous buffers	Reduce flow rate.
No target protein eluted	No His-tagged protein	Confirm expression via Western Blot.
	Low expression	Optimize expression conditions.
	Weak binding	Increase Wash Buffer pH or reduce imidazole.
	Strong binding	Lower elution pH or increase imidazole.
Protein degradation	Proteolysis	Purify at 4 °C. Add protease inhibitors.
Impure eluate	Incomplete wash	Increase Wash Buffer volume.
	Contaminant His-proteins	Optimize wash pH/imidazole. Use orthogonal chromatography.
Protein precipitation	High temperature	Load at 4 °C.
	Aggregation	Add 0.1% Triton X-100/Tween-20 to buffers.

