**ProteinIso® Ni-NTA Resin**

Cat. No. DP101

**Storage:** at 2-8°C (20% ethanol) for two years

**Description**

*ProteinIso®* Ni-NTA Resin allows rapid affinity purification of His-tagged proteins. The His-tagged proteins bind to Ni²⁺ cations, which are immobilized on the Ni-NTA resin by 4 metal-chelating sites. After wash, the target proteins are recovered by gradient elution. The resin can be used for both native and denatured protein purification.

### Resin Specifications

<table>
<thead>
<tr>
<th>Resin</th>
<th>Cross-linked 6% agarose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>NTA</td>
</tr>
<tr>
<td>Shape</td>
<td>sphere</td>
</tr>
<tr>
<td>Pore size</td>
<td>45–165 μm</td>
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<tr>
<td>Binding capacity</td>
<td>10–20 mg/ml wet gel</td>
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<tr>
<td>Recommended flow rate</td>
<td>&lt;300 cm/h</td>
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<tr>
<td>Highest resistance of atmospheric pressure</td>
<td>0.3 Mpa</td>
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<tr>
<td>pH stability</td>
<td>3-13</td>
</tr>
</tbody>
</table>

**Procedures**

1. **Prepare Ni-NTA purification column**
   1.1 Thoroughly resuspend the Ni-NTA resin to achieve a homogeneous suspension of the resin in the 20% ethanol storage buffer.
   1.2 Immediately transfer the resin into a purification column. Ensure that the bottom of the column is plugged with a stopper.
   1.2.1 Close the valve of the column. Allow the resin to settle.
   1.3 Equilibrate the column with 5–10 bed volume of equilibration buffer.

2. **Prepare samples**
   - To avoid blocking column, samples should be centrifuged and filtrated with 0.45 μm filter before loading.

3. **Load samples and wash**
   - Load samples and wash with 5–10 bed volume of equilibration buffer and collect the flow-through in one tube.

4. **Elute**
   - Elute target proteins with imidazole or low pH buffer.

5. **Regeneration of Ni-NTA resin**
   5.1 Wash the column/resin with 2 bed volume of 6 M GuHCl, 0.2 M acetic acid
   5.2 5 bed volume of deionized water
   5.3 3 bed volume of 2% SDS
   5.4 1 bed volume of 25% ethanol
   5.5 1 bed volume of 50% ethanol
   5.6 1 bed volume of 75% ethanol
   5.7 5 bed volume of 100% ethanol
   5.8 1 bed volume of 75% ethanol
   5.9 1 bed volume of 50% ethanol
   5.10 1 bed volume of 25% ethanol
   5.11 1 bed volume of deionized water
   5.12 5 bed volume of 100 mM EDTA, pH 8.0
   5.13 10 bed volume of deionized water
   5.14 5 bed volume of 100 mM NiSO₄
   5.15 Store column/resin in 20% ethanol.
Notes

- Samples should be centrifuged and filtrated with 0.45 µm filter before loading.
- Equilibration Buffer for soluble protein
  300 mM NaCl, 50 mM sodium phosphate buffer, 10 mM imidazole, 10 mM Tris-Cl pH 8.0
- Equilibration Buffer for inclusion body
  6 M GuHCl, 100 mM sodium phosphate buffer, 10 mM Tris-HCl pH 8.0; or 8 M urea, 100 mM sodium phosphate buffer, 10 mM Tris-HCl pH 8.0