PRODUCT INFORMATION

Catalog No. : G0201, G0202 and G0203

Product Name: EZ Ni-Agarose 6 Resin

Size:	G0201	5 ml
	G0202	25 ml
	G0203	100 ml

Description: EZ Ni-Agarose 6 Resin is widely used for purification of proteins with an affinity tag of six consecutive histidine residues—the 6xHis tag. Because of its remarkable binding selectivity for 6xHis tag, EZ Ni-Agarose 6 Resin makes one-step purification possible for almost any His-tagged protein, from any expression system, under native or denaturing conditions. The EZ Ni-Agarose 6 Resin is supplied precharged with Ni^{2+} ions. Proteins with 6xHis tag bind to the resin. Impurities such as unwanted proteins will wash away, and purified His-tagged proteins can be eluted by imidazole or reduced pH solution.

Imidazole at low concentrations can be included in binding and wash buffers to minimize the non-specific binding of unwanted host cell proteins. However, imidazole may also decrease the binding of histidine-tagged proteins. Therefore, the imidazole concentration must be optimized to ensure the best balance of high purity and high yield. Optimal concentration of imidazole is protein-dependent and therefore must to be determined by experiment.

Storage and Precautions:

The resin is stable at least for one year. The resin must be cleaned after each use and stored in 20% ethanol, which should be removed by washing with water before use (see below). Store the resin at 2-8^{o}C.

Note: This Product Is For Research Use Only.

Protocol for His-tagged Proteins using EZ Ni-Agarose 6 Resin

Buffers and solutions supplied by user:

Buffer A: 20 mM potassium phosphate
500 mM potassium chloride
10 mM imidazole pH 7.5.

Buffer B1: 20 mM potassium phosphate pH 7.5.

Buffer B2: 20 mM potassium phosphate
500 mM imidazole pH 7.5.

Elution buffer: Make fresh each time by mixing Buffer B1 and Buffer B2 according to your needs (see below).

Notes:

1. The buffers above are recommended for binding, washing, and eluting Histidine-tagged recombinant proteins using batch or gravity-flow procedures. However, the imidazole concentration may need to be optimized for each individual Histidine-tagged protein. For most Histidine-tagged proteins, an imidazole concentration of 10–20 mM in the binding and wash buffers is a good starting point.
2. The following protocols use 1 gram of cell paste as an example; increase buffers proportionally to your sample volumes. Typically, 1 liter of an overnight culture of bacteria will yield 3 grams of cell paste.
3. Sodium phosphate and sodium chloride can be substituted for potassium phosphate and potassium chloride in all buffers and solutions.

Sample preparation:

1. Harvest host cells with expressed Histidine-tagged protein.
2. Lyse host cells by adding lysozyme (0.2 mg/ml) and sonicating in 8 ml of Buffer A per gram cell paste.
3. Clarify lysate by centrifugation at 10,000 ×g for 10 min.
4. Filter lysate through a 0.45-µm filter.

COLUMN PURIFICATION WITH EZ NI-AGAROSE 6 RESIN

1. Gently shake the bottle in which the EZ Ni-Agarose 6 Resin is supplied until the medium is homogeneous.
2. Transfer 2ml of resin slurry from the bottle into a suitable chromatographic column, for example, a plastic column 5cm long and 1cm diameter.
3. Sediment the EZ Ni-Agarose 6 Resin by gravity.
4. Wash the EZ Ni-Agarose 6 Resin with 5ml distilled water.
5. Wash the EZ Ni-Agarose 6 Resin with 5ml Buffer A.
6. Load the sample prepared in last section to the EZ Ni-Agarose 6 Resin.
7. Collect the sample flowthrough.
8. Wash the EZ Ni-Agarose 6 Resin four times with 5ml Buffer A.
9. Elute the protein with Elution Buffer by gradient elution* or column step elution**.
10. Evaluate the protein in flowthrough from Step 7 and each sample elution from Step 9 by protein electrophoresis.

***Gradient Elution:** use **Buffer B1** as low concentration elution buffer and **Buffer B2** as high concentration elution buffer. Depending on the nature of specific proteins, the optimal concentration of imidazole could vary.

****Column Step Elution:** mix **Buffer B1** and **Buffer B2** to prepare several different **Elution buffers**; we suggest imidazole concentrations such as 25mM, 50mM, 100mM 250mM and so on. Use 2ml of each Elution Buffer to elute proteins from the column. The optimal concentration of imidazole differs from protein to protein, but most will elute somewhere between 50mM

and 300mM imidazole.

Batch Purification with EZ Ni-Agarose 6 Resin

1. Gently shake the bottle in which the EZ Ni-Agarose 6 Resin is supplied until the medium is homogeneous.
2. Transfer 2ml of resin slurry from the bottle to a centrifuge tube.
3. Sediment by centrifugation at 500 ×g for 5 min.
4. Discard supernatant and replace with 5 ml distilled water.
5. Shake gently for 3 min and resediment by centrifugation at 500 ×g for 5 min.
6. Discard supernatant and resuspend with 2ml Buffer A.
7. Mix with sample prepared in last section.
8. Incubate at room temperature for 30 min on a shaker at low speed.
9. Sediment by centrifugation at 500 ×g for 5 min.
10. Collect the supernatant and replace with 5ml Buffer A.
11. Shake gently for 3 min and resediment by centrifugation at 500 ×g for 5 min.
12. Discard supernatant and replace with 5ml Buffer A.
13. Repeat Steps 11 and 12 two more times.
14. Elute the protein with Elution Buffer by batch step elution***.
15. Evaluate the protein in each elution and the sample supernatant from step 10 by protein electrophoresis.

***Batch step elution:** mix **Buffer B1** and **Buffer B2** to prepare several different **Elution buffers**; we suggest imidazole concentrations such as 25 mM, 50 mM, 100 mM 250 mM and so on. After step 13, resuspend the resin in 2ml of the lowest concentration of **Elution Buffer**, sediment by centrifugation at 500 ×g for 5 min, collect the supernatant. Repeat the steps with each increasing concentration of **Elution Buffer**. The optimal concentration of imidazole differs from protein to protein, but most will elute somewhere between 50 mM and 300 mM imidazole.

Re-Charging the Resin/Storing the Column

1. Wash the column with 2 volumes of Regeneration Buffer (6 M GuHCl, 0.2 M acetic acid).
2. Wash the column with 5 volumes of water.
3. Wash the column with 3 volumes of 2% SDS.
4. Wash the column with 1 volume of 25% ethanol.
5. Wash the column with 1 volume of 50% ethanol.
6. Wash the column with 1 volume of 75% ethanol.
7. Wash the column with 5 volumes of 100% ethanol.
8. Wash the column with 1 volume of 75% ethanol.
9. Wash the column with 1 volume of 50% ethanol.
10. Wash the column with 1 volume of 25% ethanol.
11. Wash the column with 1 volume of water.

12. Wash the column with 5 volumes of 100 mM EDTA, pH 8.0.
13. Wash the column with water.
14. Recharge the column with 2 volumes of 100 mM NiSO4.
15. Wash the column with 2 volumes of water.
16. Wash the column with 2 volumes of Regeneration Buffer.
17. Equilibrate with 2 volumes of a suitable buffer (eg. Buffer A, or Buffer B).

Notes:

After use, the columns should be washed for 30 minutes with 0.5 M NaOH, and stored in 30% ethanol to inhibit microbial growth. We recommend a maximum of 5 runs per column.

Technical Tips

Optimization:

1. You may use Tris-HCl buffer in sample preparation, wash buffer or elution buffers. However, when protein binding is weak, Tris buffers should be avoided.
2. Do not use chelating agents such as EDTA and citrate in any working buffer.
3. Do not use hydrogenocarbonate or carbonate containing buffers.
4. High salt concentration (NaCl or KCl) up to 1.5 M can be used for more stringent washing.
5. Higher concentration of imidazole can also be used for possible non-specific binding.

Reagents compatible with EZ Ni-Agarose 6 Resin:

2% Triton X-100, 2% Tween 20, 1% CHAPS, 20 mM β-ME, 50% glycerol,
2 M NaCl, 6 M guanidine, 8 M Urea

Troubleshooting

Column has clogged:

- There may be cell debris in the sample: centrifuge and/or filter the sample through a 0.22 μm or a 0.45 μm filter (see Sample preparation).
- If the sample is very viscous, host nucleic acid may be the cause. Sonicate until the viscosity is reduced, and/or add 5 μg/ml DNase I, Mg^{2+} to 1 mM, and incubate on ice for 10–15 minutes. Alternatively, draw the lysate through a syringe needle several times.

Histidine-tagged protein found in the pellet:

- Sonication may be insufficient. Addition of lysozyme prior to sonication may improve results.
- Avoid frothing and overheating as this may denature the target protein.
- The protein may be insoluble (inclusion bodies): The protein can usually be solubilized (and unfolded) from inclusion bodies using common denaturants such as 4–6 M Gua-HCl, 4–8 M urea or strong detergents.

Histidine-tagged protein is found both in the flow-through / supernatant and elution fractions:

- Capacity of Ni Sepharose 6 Fast Flow is exceeded: Increase the volume of Ni Sepharose 6 Fast Flow used for your purification.

No histidine-tagged protein is found both in the elution fractions

- Elution conditions may be too mild. Elute with an increasing imidazole gradient or decreasing pH to determine the optimal elution conditions.
- The protein has precipitated in the column: Try detergents or changed NaCl concentration or elute under denaturing (unfolding) conditions to remove precipitated proteins.
- Verify DNA sequence of the gene. Analyze samples taken before and after induction of expression with, e.g., anti-His antibodies in Western blotting.
- Histidine-tag may be insufficiently exposed. Perform purification in urea or Guanidine-HCl as for inclusion bodies.
- Buffer/sample composition is incorrect. Check pH and composition of sample and binding buffer. Ensure that the concentration of chelating or strong reducing agents, as well as imidazole, in the solution is not too high.

The eluted protein is not pure (multiple bands on SDS polyacrylamide gel)

- Partial degradation of tagged protein by proteases: Add protease inhibitors.
- Optimize imidazole concentration for binding buffer.
- Add detergent and/or reducing agents before sonicating the cells. Increase the detergent levels (e.g. up to 2% Triton X-100 or 2% Tween 20), change the NaCl concentration or add glycerol (up to 50%) to the wash buffer.

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