Anti-His Affinity Resin

Technical Manual No. TM0641 **Version 05102013**

Product Name	**Cat.No.**	**Size**
Anti-His Affinity Resin	L00439-1	1 ml
	L00439-5	5 ml

The product is designed for purification and immunoprecipitation of His-tagged protein, especially for the sample failed by Ni-NTA agarose chromatography purification.

The operator should read technical manual carefully before using this product.

For research use only. Not for diagnostic use.

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I. Product Description and Features

His tag consists of successive histidine (H) residues which mainly contains HHHHHH (6 x His), HHHHH (5 x His) and HHHH (4 x His). Due to its small size, less interference in protein folding and weak immunogenicity, His tag is the most commonly used tag in recombinant protein expression. A DNA sequence which codes for His tag, is usually constructed at N-terminus or C-terminus of various expression plasmids. Anti-His tag antibody is a useful tool for the analysis of His-tagged proteins with different applications such as western blot, immunoprecipitation and flow cytometry.

Ni-NTA Agarose chromatography is a common tool to purify His-tagged protein from *Ecoli*, yeast or mammalian cell expression system by one step purification. However, it is difficult to get His-tagged protein with high purity by using Ni-NTA Agarose when non-specific proteins bind to the resin tightly and fail to be removed completely by washing. Sometimes, it is also hard for Ni-NTA Agarose to purify His-tagged proteins, especially when His-tagged protein is present as a small fraction in samples. To solve this problem, Anti-His Affinity Resin is a perfect alternative. It is an immunoaffinity resin with THE™ anti-His monoclonal antibody, which can bind His-tagged proteins in sample. THE™ anti-His monoclonal antibody has high affinity to His tag so the resin can bind with His-tagged protein at low level in the samples. Combination of Ni-NTA Agarose with Anti-His Affinity Resin contributes to high purity of His-tagged protein purification. Case studies demonstrated that His-tagged proteins which were hard to be purified by Ni-NTA Agarose, could be highly purified with Anti-His Affinity Resin.

GenScript Anti-His Affinity Resin is designed for purification of His-tagged protein that is difficult for single Ni-NTA Agarose purification. This product is made from THE™ anti-His monoclonal antibody conjugated to agarose resin. It specifically binds to His-tagged proteins in the samples. Proteins nonspecifically binding to the resin can be eliminated by washing. Finally, His-tagged protein can be eluted by different methods. In addition to that, the resin can be used for immunoprecipitation of His-tagged proteins.

Table 1. Characteristics of Anti-His Affinity Resin

Feature	Specification
Product content	50% resin in TBS with 0.02% sodium azide
Beads structure	4% agarose
Beads size	90 µm
Coupled antibody	THE™ His Tag Antibody, mAb, Mouse (Cat.No A00186)
Binding capacity	More than 0.6 mg of His-tagged protein per ml of settled resin
Specificity	N-terminal/C-terminal/internal /4 x His/5 x His/6 x His-tagged proteins
Storage and stability	Stable at 2-8 °C for up to 12 months. Do not freeze the resin
Repeat usage	Generally the resin can be recycled at least 4 times. If maintained properly, the resin may reach 10 recycle times with minor binding capability loss
Elution method	Acid, alkaline and peptide competitive elutions
Reagents compatibility	Compatible with mostly used reagents at certain concentrations
Consistency	Lot-to-lot consistency proved by strict production procedure

II. Equipments and Reagents Required But Not Supplied

Distilled water; Microcentrifuge tube; Reagent reservoir dishes; Column; Pipette; His-tagged protein sample; Protease inhibitor reagent;

Table 2. Buffer solutions for purifying His-tagged proteins

Purpose	Buffer	Formulation
Equilibration and Washing	Tris-buffered saline, TBS	50 mM Tris-HCl, 150 mM NaCl, pH 7.4
Elution	Alkaline elution buffer	0.1 M Tris, 0.5 M NaCl, pH 12.0
	Acid elution buffer	0.1 M Glycine HCl, pH 2.5
	His peptide buffer	His peptide (HHHHHH) in TBS with concentration of 0.5-1 mg/ml
	SDS-PAGE loading buffer	0.01 M Tris-HCl, 1% SDS, 10% Glycerol, 0.016% Bromophenol Blue
Neutralization	Alkaline neutralization buffer	1 M HCl
	Acid neutralization buffer	1 M Tris, pH 9.0
Resin Regeneration	Regeneration buffer A	0.1 M Tris HCl, 0.5 M NaCl, pH 8.0
	Regeneration buffer B	0.1 M Sodium acetate, 0.5 M NaCl, pH 4.0
Storage	Storage buffer	TBS with 0.02% sodium azide

III. Protocol

1. Sample Preparation

When preparing samples for purification, several tips should be considered as below.

- Prepare the samples according to the nature of the target protein and minimize the concentration of reagents in the samples which could interfere with His-tagged protein binding to Anti-His Affinity Resin (*see IV. Reagent Compatibility Table*).
- A proper concentration of NaCl (0.15 M) and neutral pH (pH 7.4) is required for the His-tagged protein binding. The samples should not contain any particles. Treat the samples with 0.22 µm filter or centrifuge with 10, 000 rpm for 10-15 min at 4 °C to remove the insoluble materials before the binding procedure.
- Protease inhibitor might be added to the samples if needed. Keep the samples on ice to prevent the target protein from degradation.
- If the samples containing chromosomal nucleic acids are viscous, nuclease should be added to degrade the nucleic acids.
- Avoid repeated freezing and thawing cycles. Aliquot the samples and store at -80 °C if necessary.

2. Affinity Resin Preparation

- Calculate a required amount of the resin according to the His-tagged protein amount in the sample.
- End-cutted pipette is recommended to transfer the resin slurry to prevent it from clogging.
- Do not leave the resin without soaking.

2.1 Rinse the empty column with 2-3 x resin-volume of TBS.

2.2 Thoroughly resuspend the resin to form the slurry and load an appropriate volume of the resin into the column.

2.3 Wash the resin with 2-3 x resin-volume of TBS, and drain the buffer from the resin after washing.

3. Procedure of His-tagged Protein Binding

- Column procedure and batch procedure can be opted for His-tagged protein binding.
- For large volume samples (>50 ml), the batch procedure is recommended for the protein binding.
- Low flow rate may facilitate the His-tagged protein binding.
- Keep the flow-through for further SDS-PAGE or Western Blot assay.

3.1 Column Procedure

3.1.1 Load the prepared sample onto the column at room temperature and collect the flow-through with a tube.

3.1.2 The flow-through is reloaded for 2-3 times onto the column to reach maximal binding. Keep the flow-through for further use.

3.1.3 Wash the resin with 10-20 x resin-volume of TBS to remove any non-specific binding materials.

3.1.4 Allow the resin drain out and apply the elution buffer quickly (See *4. Elution of His-tagged protein*).

3.2 Batch Procedure

3.2.1 Resuspend the prepared resin from the column and transfer it into a tube.

3.2.2 Incubate the sample with the resin at room temperature for at least 30 mins with rotation.

3.2.3 Load the sample with the resin onto the column and collect the flow-through in a new tube for further use.

3.2.4 Wash the resin with 10-20 x resin-volume of TBS to remove any non-specific binding materials.

3.2.5 Allow the resin drain out and apply the elution buffer quickly (See *4. Elution of His-tagged protein*).

4. Elution of His-tagged Protein

- Several elution buffers can be used to separate the His-tagged protein from the resin (*See II*. Equipments and Reagents Required But Not Supplied).
- An optimal elution buffer for eluting a unique His-tagged protein should be decided empirically. The optimal elution method should keep maximal protein structure integrity and maintain the function, as well as to be applicable for downstream procedures.
- His-tagged protein can be eluted efficiently from the resin by alkaline elution buffer.
- Do not leave the resin in alkaline elution buffer or Acid elution buffer for more than 15 mins.
- All the methods could be carried out at room temperature. Low temperature might be needed depending on special requirement.

4.1 Alkaline Elution Procedure

4.1.1 Label six tubes and add 1/20 resin-volume of 1 M HCl into each tube.

4.1.2 Add 6 x resin-volume of the alkaline elution buffer to the column to separate the His-tagged protein from the resin.

4.1.3 Collect the 1 x resin-volume of the eluate from the column into each tube containing 1 M HCl.
For example, if 1 ml of settled resin is applied, 6 ml of elution buffer is used to elute His-tagged protein and collect 1 ml of eluate per tube containing 50 µl of 1 M HCl, respectively.

4.1.4 Wash the resin with 5-10 x resin-volume of TBS.

4.2 Acid Elution Procedure

4.2.1. Label six tubes and add 1/20 resin-volume of 1 M Tris into each tube.

4.2.2. Add 6 x resin-volume of the Acid elution buffer to the column to elute the His-tagged protein from the resin.

4.2.3. Collect 1 x resin-volume of the eluate from the column to each tube containing 1 M Tris.
For example, if 1 ml of settled resin is applied, 6 ml of elution buffer is used to elute His-tagged protein and collect 1 ml of the eluate per tube containing 50 µl of 1 M Tris, respectively.

4.2.4. Wash the resin with 5-10 x resin-volume of TBS.

4.3 Competitive Elution Procedure

4.3.1. His peptide is used to elute His-tagged protein from the resin through competitive binding.

4.3.2. Dissolve the His peptide in a tube to prepare His peptide solution. His peptide solution with concentration of 0.5-1 mg/ml can be used for His-tagged protein elution.

4.3.3. Add 2-3 x resin-volume of His peptide solution to the resin.

4.3.4. Incubate the resin with shaker at room temperature for 30-60 mins.

4.3.5. Load it into the column and collect the eluate containing His-tagged protein in a vial.

4.3.6. Wash the resin with 2-3 x resin-volume of Acid elution buffer to remove His peptide from the resin

4.3.7. Wash the resin with 5-10 x resin-volume of TBS.

5. Regeneration of Affinity Resin

- The resin can be reused to purify the same protein for several times without regeneration. If purify a different His-tagged protein with the recycled resin, the resin must be regenerated as below.

5.1. Wash the resin with 2 x resin-volume of Regeneration buffer A.

5.2. Wash the resin with 2 x resin-volume of Regeneration buffer B.

5.3. Wash the resin with 5-10 x resin-volume of TBS.

5.4. Store the resin in TBS containing 0.02% sodium azide at 2-8 °C.

6. Immunoprecipitation of His-tagged Proteins

- End-cutted pipette is recommended to transfer the resin slurry to prevent it from clogging.
- Carefully remove the supernatant as much as possible after each washing without disturbing the settled resin.

6.1. Resuspend the resin to form uniform slurry and transfer 40-100 µl of slurry into a 1.5 ml tube.

6.2. Add 500 µl of PBS into the resin and gently mix it. Centrifuge at 10, 000 rpm for 60 seconds at 4 °C and remove supernatant carefully with pipette. Repeat the washing step twice.

6.3. Add 100 µl of sample to the tube containing the resin and gently mix it. Rotate the tube on a shaker for at least 1 hour at room temperature.

6.4. Centrifuge the resin at 10, 000 rpm for 60 seconds at 4 °C. Carefully remove the supernatant with pipette.

6.5. Add 500 µl of PBST into the resin and gently mix it. Centrifuge at 10, 000 rpm for 60 seconds at 4 °C and remove supernatant carefully with pipette. Repeat the washing step for three times.

6.6. Add 40 µl of SDS-PAGE loading buffer to the tube and mix it thoroughly.

6.7. Heat the tube at 90-100 °C for 10 mins.

6.8. Centrifuge at 10,000 rpm for 60 seconds at 4 °C to spin down the resin.

6.9. Collect the supernatant carefully and perform SDS-PAGE analysis immediately or store it at -80 °C.

7. Detection and Identification

SDS-PAGE and Western Blot analysis are commonly used methods to evaluate purification efficiency. After purification, SDS-PAGE and Western Blot assay could be set up to detect the purified proteins as well as starting materials, such as raw cell lysate, \supernatant, and flow-through fractions. High yield of His-tagged proteins could be loaded directly onto SDS-PAGE gel for detection. Low yield His-tagged protein is sometimes hard to be detected by SDS-PAGE. In this case, Western Blot is suggested, or purified His-tagged protein could be firstly concentrated, and then loaded onto the SDS-PAGE gel for detection. The SDS-PAGE and Western Blot procedures are specified elsewhere, please go to www.GenScript.com for detailed information.

IV. Reagent Compatibility

Table 3. Reagent compatibility

Reagent	Maximum Tolerable Concentration	Notes
EDTA	10 mM	Higher concentration of chelating agent may impair protein purification.
β-ME	10 mM	Higher concentration may impair the coupled antibody.
DTT	10 mM	
Tween 20	1%	Higher concentration may interfere with the protein binding.
Triton X-100	5%	Higher concentration may interfere with the protein binding.
SDS	0.1%	Higher concentration may denature the coupled antibody.
NP-40	1%	Higher concentration may impair the protein binding.
CHAPS	1%	Higher concentration may interfere with the protein binding.
Urea	1 M	Higher concentration may denature the coupled antibody.
Glycerol	10%	Higher concentration may interfere with the protein binding.
NaCl	1 M	Higher concentration may impair the protein binding.

V. Test Results

Description	Picture	Figure legend
Figure 1. His-tagged protein purified by Anti-His Affinity Resin or Ni-NTA Agarose	Sample A, Sample B; 200 kDa, 97.2 kDa, 66.4 kDa, 44.3 kDa, 29.0 kDa, 20.1 kDa, 14.3 kDa; Purified by Anti-His Affinity Resin / Sample A, Sample B; 200 kDa, 97.2 kDa, 66.4 kDa, 44.3 kDa, 29.0 kDa, 20.1 kDa, 14.3 kDa; Purified by Ni-NTA Agarose	SDS-PAGE analysis of protein purified by Anti-His Affinity Resin or Ni-NTA Agarose. Test result demonstrated that the Anti-His Affinity Resin can purify the His-tagged proteins with high purity from different samples in which Ni-NTA Agarose had failed.
Figure 2. His-tagged proteins eluted by different elution solutions	M, 1, 2, 3; 200 kDa, 116 kDa, 97.2 kDa, 66.4 kDa, 44.3 kDa, 29.0 kDa	SDS-PAGE analysis of protein eluted from Anti-His Affinity Resin. M. Marker 1. His-tagged protein using the acid elution procedure 2. His-tagged protein using the alkaline elution procedure 3. His-tagged protein by competitive elution procedure Test result demonstrated that the His-tagged protein can be eluted by three different eluents.

VI. Troubleshooting

Problem	Probable Cause	Solution
No binding or less binding	Binding time is not enough	If using batch method, increase the binding time. If using column method, use a lower flow rate to improve the binding efficiency.
	His-tag is not accessible to resin	Denature the protein or switch His tag to the other terminus of the protein.
	Resin needs regeneration after purification	Regenerate the resin according to the procedure described in manual.
	Reagents compatibility problem	Dialyze the sample before the purification procedure.
Minimal or no His-tagged protein present in the elution fraction	Protein degradation	Perform purification at 4 °C and add protease inhibitors to the samples during the binding step. Avoid using frozen samples and prepare fresh sample.
	Protein is not fully eluted	Change the elution methods described in manual.
	Protein is not expressed	Check the sample for the presence of His-tagged protein by Western blot before purification.
	Protein expression is very low	Add more samples or optimize the expression conditions to increase the yield.
	Elution method is not suitable	Try different elution method.
	Low concentration of His-tagged proteins in the sample	Concentrate the elution fraction before SDS-PAGE test.
His-tagged protein appears as multiple bands on stained gels	The protein is not stable	Purify the protein at 4 °C.
	Proteolytic activity occurs during the purification	Add protease inhibitors to samples and wash buffers.
	Non-specific binding	Prepare the sample again or add additional wash steps.

VII. Related Products

THE™ His Tag Antibody, mAb, Mouse	A00186
THE™ His Tag Antibody [HRP], mAb, Mouse	A00612
THE™ His Tag Antibody [Biotin], mAb, Mouse	A00613
THE™ His Tag Antibody [FITC], mAb, Mouse	A01620
His-Tag Antibody, pAb, Rabbit	A00174
His-Tag Mini Purification and Detection Kit	L00438
His Tag ELISA Detection Kit	L00436
His Tag Antibody Plate	L00440
Ni-charged MagBeads	L00295
Mouse Anti-His mAb MagBeads	L00275

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