Ni-NTA Agarose Resin

DS0821 – 5ML
DS0821 – 25ML

Ni-NTA Agarose Resin uses nitrilotriacetic acid (NTA), a tetradentate chelating ligand, in a highly cross- linked 6% agarose matrix. NTA binds Ni2+ ions by four coordination sites. His-tagged proteins expressed in E. coli can be purified and detected easily because the histidine residues bind metal ions like nickel immobilized on a column matrix under specific buffer conditions and bound proteins are eluted out by changing the pH or by adding a competitive molecule like imidazole.

HiMedia's Ni-NTA Agarose Resin supplied as a 50% slurry suspension in 30% ethanol is used for rapid purification of recombinant proteins fused to 6X HIS and expressed in bacteria.

1 ml resin corresponds to 2 ml of 50 % (v/v) Ni-NTA Agarose slurry suspension.

Recommendations: The yield of His-tagged protein depends upon various parameters, such as amino acid composition, 3-D structure, molecular weight, etc.

Ni-NTA Agarose Resin has an orientative binding capacity of up to 50 mg/ml resin.

I. Procedure for Batch Purification of His-Tagged Proteins under native conditions

1. Elimination of the Preservative: Determine the quantity of Nickel NTA Agarose Resin needed for purification following the recommendations. Gently shake the bottle of Ni- NTA Resin to achieve a homogenous suspension. Immediately pipette the suspension (2 ml of the original Ni-NTA agarose suspension per ml of resin volume required) to an appropriate tube, sediment the resin by centrifugation at 500 x g for 5 minutes and carefully decant the supernatant and discard it.
2. Equilibration of the Resin: Add 10 bed volumes of binding buffer to equilibrate the resin by mixing thoroughly to achieve a homogenous suspension. Sediment the resin by centrifugation at 500 x g for 5 minutes and carefully decant the supernatant and discard it.
3. Application of the Sample: Once the resin is equilibrated, the sample containing the fused protein for purification is applied. In some cases, a slight increase of contact time may facilitate binding. Centrifuge the suspension at 500 x g for 5 minutes to sediment the resin and carefully decant the supernatant and discard it.

   Note: The choice of binding buffer depends on the particular properties of the protein. The buffer used most frequently is phosphate (50 mM is recommended). The pH of binding buffers generally leads to neutrality (7.0 – 8.0). Binding capacity can be affected by several factors, such as sample concentration, binding buffer or the flow rate during sample application.

   Collect flow through and verify that fused protein has bound.

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4. Washing of the Resin: Wash the resin by adding 10 ml be volumes of wash buffer. Invert to mix and centrifuge the suspension at 500 x g for 5 minutes to sediment the resin. Carefully decant the supernatant and discard it. Repeat the washing step twice.
5. Elution of Pure protein: Add 1 bed volume of elution buffer to the resin. Mix thoroughly for 10 minutes at room temperature. Sediment the resin by centrifugation at 500 x g for 5 minutes and carefully decant or pipette the supernatant in a new tube and store on ice. Repeat the elution step twice or more and pool the fractions.

Note: It is possible that a significant amount of HIS-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among His-tagged proteins. Eluates should be monitored by Bradford assay, SDS-PAGE or by measuring the absorbance at 280 nm.

II. Procedure for Gravity Purification of His-Tagged Proteins under native conditions

1. Elimination of the preservative: Determine the quantity of Ni-NTA resin needed for purification. Gently shake the bottle of resin to achieve a homogenous suspension. Immediately pipette sufficient suspension to an appropriate empty column. Remove first the upper cap and then the lower one of the columns to allow elimination of the preservative by gravity flow.
2. Equilibration of the Pre-packed column: Equilibrate the column with 5X resin bed volume of binding buffer and allow draining the buffer through column. Do not let the resin bed dry.
3. Application of the Sample: Close the bottom cap and add the sample containing the His-tagged protein to be purified (clarified *E. coli* lysate) through the top of the column. Close the top cap and keep sample and resin in contact for at least 45 - 60 minutes before removing the bottom cap. Collect the flow through.
4. Washing: Close the bottom cap. Add 5X bed volumes of the wash buffer through the top to eliminate all the proteins that have not been retained in the column. Close the top cap and mix manually inverting the column. Remove the bottom cap and discard the flow through. Repeat the step twice.

   Note: Wash the column until the absorbance at 280 nm of the eluent reaches the baseline.

   Optional: Keep all the washes if required.
5. Elution of the pure protein: Close the bottom cap and add the elution buffer through the top. Close the top cap and mix manually inverting the column thoroughly for 15 minutes. Let the resin settled, remove the bottom cap and collect the eluate in a new tube and store on ice. Repeat the elution step twice and pool the collected eluates.

   Note: It is possible that a significant amount of His-tagged protein may remain bound to the resin.

Conditions (volumes, times, temperatures) used for elution may vary among His-tagged proteins. Eluates should be monitored by Bradford assay, SDS-PAGE or by measuring the absorbance at 280 nm.

III. Procedure for Spin Purification of His-Tagged Proteins under native conditions

1. Elimination of the preservative: Determine the quantity of Ni-NTA resin needed for purification. Gently shake the bottle of resin to achieve a homogenous suspension. Immediately pipette 100 µl of the original suspension to the empty spin column. Remove the lower cap of the column and place it in the 2 ml collection tube. Centrifuge at 500 X g for 1 minute to allow elimination of the preservative.
2. Equilibration of the spin column: Equilibrate the spin column with 0.4 ml of binding buffer and mix manually. Centrifuge at 500 X g for 1 minute and discard the flow through. Repeat this step once. Do not let the resin bed dry.
3. Application of the Sample: Close spin column outlet with cap and add the sample containing the His- tagged protein to be purified (clarified *E. coli* lysate) through the top of the spin column. Close the lid and keep sample and resin in contact for at least 30 minutes before removing the bottom cap. Mix manually inverting the spin column. Centrifuge at 500 X g for 1 minute and collect the flow through.
4. Washing: Transfer the spin column to a new collection tube. Add 0.4 ml of washing buffer through the top to eliminate all the proteins that have not been retained in the column. Mix manually inverting the spin column. Centrifuge at 500 X g for 1 minute and discard the flow through. Repeat the washing step twice for a total of three washes.
   Note: Wash the column until the absorbance at 280 nm of the eluent reaches the baseline.
   Optional: Keep all the washes if required.
5. Elution of the pure protein: Transfer the spin column to a new collection tube and close the column outlet with cap. Add 0.4 ml of elution buffer and close the lid. Mix thoroughly for 10 minutes before removing the bottom cap. Centrifuge at 500 X g for 1 minute, collect the eluate and label it. Repeat the elution step twice for a total of three individual eluates.

   Note: It is possible that a significant amount of His-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among His-tagged proteins. Eluates should be monitored by Bradford assay, SDS-PAGE or by measuring the absorbance at 280 nm.

IV. Procedure for FPLC Purification of His-Tagged Proteins under native conditions

Ni-NTA Agarose Resin is compatible with common low-pressure chromatography columns and FPLC applications. We recommend columns equipped with an adjustable plunger/flow adapter. Use low rates for loading step to allow maximal binding of the His-tagged protein.

1. Column Packaging: Manually shake the bottle to obtain a homogenous suspension of Ni-NTA Agarose Resin. Place a funnel in the head of column and slowly run the suspension down the walls of the column. Insert the adapter gently in the column head until it begins to displace the liquid. Make sure no air is trapped under the net.

   Note: It is advisable to make the addition slowly to avoid the formation of bubbles. The product may also be degassed before adding to the column.
2. Elimination of the preservative: Add distilled water to the purification stream until a constant height is achieved. When a constant height has been obtained, maintain the flow with the addition of 5 volumes of distilled water to completely eliminate the preservative.
3. Equilibration of the column: Equilibrate the column with at least 5 column volumes of binding buffer until the OD 280 nm is stable.

   Note: It is advisable to previously de-gas at the solutions before adding to the column to avoid the formation of bubbles.
4. Application of the Sample: Once the resin is equilibrated, the sample containing the fused protein for purification is applied. In some cases, a slight increase of contact time may facilitate binding. Therefore, low rates can be used for the loading step to allow maximal binding of the His-tagged protein.

   Note: Binding capacity can be affected by several factors, such as sample concentration, binding buffer or the flow rate during sample application. Collect flow through and verify that fused protein has bound.
5. Washing of the Resin: Wash the resin by adding 10 - 20 bed volumes of wash buffer or until the OD 280 nm reaches the baseline level.
6. Elution of Pure protein: Elute the His-tagged protein with 5 -10 bed volumes of Elution buffer and collect the fractions on ice.

   Note: It is possible that a significant amount of His-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among His-tagged proteins. Eluates should be monitored by Bradford assay, SDS-PAGE or by measuring the absorbance at 280 nm.

Additional Information:

If the expressed proteins form inclusion bodies then the purification procedure has to be carried out in denaturing condition using 8M Urea or 6M Guanidine hydrochloride.
For denaturing conditions, the following buffers can be used:

Denaturing Binding Buffer: 20mM Sodium phosphate, pH 7.8, 500 mM NaCl, 8M Urea
Denaturing Wash Buffer: 20mM Sodium phosphate, pH 6.0, 500 mM NaCl, 8M Urea
Denaturing Elution Buffer: 20mM Sodium phosphate, pH 4.0, 500 mM NaCl, 8M Urea

Regeneration and Storage of Column: During the life of the resin, it may lose binding points because some protein is retained. Hence a loss of the binding capacity may be observed in successive cycles. To return to the starting state, regeneration may be necessary. Regeneration consists of the complete elimination of the retained protein. In general, column regeneration is always necessary when changing proteins. When continuing with the same protein it is recommended to do regeneration when an appreciable reduction in the yield is observed. The frequency of these stages varies with the protein and the conditions used:

- Wash the resin with 0.5M NaOH for 30 minutes.
- Remove the NaOH by washing with 10 bed volumes of distilled water.
- Wash and resuspend with 30% Ethanol. Finally, store at 2 – 8ºC.

Columns can be regenerated for at least 5 times without significant loss in binding capacity.

Troubleshooting Guide:

Sr. No.	Problem	Cause	Solution
1.	High viscosity sample	Presence of DNA in the sample/lysate	Treat the lysate with DNase I
		Presence of insoluble material in the sample/lysate	Spin or filter to avoid clogging of column.
2.	Target protein does not bind to the column	His-tag has been degraded	Use protease inhibitors and do the purification at lower temperature.
		Tag may be inaccessible	Purify the protein in denaturing conditions or add the in another site.
		Inadequate binding conditions	Check the pH for binding
3.	High amount of protein contaminants	Insufficient washing	Increase the concentration of imidazole in the buffer during washing and equilibration steps (as mentioned in Table 1).
4.	Target protein elutes poorly	Protein binding with chelating metal is too strong.	If possible, try the elution procedure at higher temperature.
		Fused protein can be precipitated	Elute in denaturing conditions and incubate the column with elution buffer for a longer period of time.

Safety Information

The HiMedia's Ni-NTA Agarose Resin is for laboratory use only, not for drug, household or other uses. Please refer the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. Ni-NTA Agarose Resin is designed and sold for research and in vitro purposes only. The product is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressed clearly for that purpose by the Food and Drug Administration or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in the text.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Symbol

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIDS0821
Rev No.: 03
Date of Issue: 2025-08