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ProductInformation

Ni-CAM® HC Resin
High Capacity Nickel Chelate Affinity Matrix

Product No. **N 3158**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Ni-CAM™ affinity resin (Ni-CAM) is an immobilized metal-ion affinity chromatography (IMAC) product. IMAC is used to affinity purify proteins containing multiple histidines or histidine clusters.[1-5] The Ni-CAM resin is a proprietary, patented, quadridentate chelate resin charged with nickel that is designed to specifically bind histidine containing proteins.[3] The matrix for this resin is 6% beaded agarose. Ni-CAM resin is selective for proteins containing histidine clusters with low non-specific binding of other proteins. The selectivity can be modulated with the inclusion of imidazole during chromatography.[5] This resin is durable and can capture the proteins at a high flow rate.[3] Proteins are bound using either native or denaturing conditions.[3] The protein capacity of this resin is 20-25 mg/ml of packed bed volume as determined with a 50 kDa protein.[3] Ni-CAM resin is supplied as a 50% suspension in 30% ethanol. **It is recommended that the entire technical bulletin be read before use, especially the reagent compatibility chart.**

Reagents and Equipment Required but Not Provided

(Product Numbers have been given where appropriate)

- Appropriate centrifuge
- CelLytic™ B Cell Lysis Reagent, Product No. B7435 or CelLytic B Cell Lysis Reagent, 2×, Product No. B7310, for bacterial lysis
- Appropriate column or centrifuge tubes
- Imidazole, Product No. I0125
- Sodium chloride, Product No. S3014
- Sodium phosphate, Product No. S0751
- Protease Inhibitor Cocktail for use in purification of histidine-tagged proteins, Product No. P8849

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Do not allow the resin to remain in any buffer for extended periods of time (>24 hours) unless some type of antimicrobial agent (20–30% ethanol) is added to the buffer.

Note: Buffers or reagents that chelate metals should not be used with this product since they may strip the metal off the column. Strong reducing agents should also be avoided since they may reduce the bound nickel and thus eliminate the binding of histidine containing proteins. See Reagent Compatibility chart for more information.

Preparation Instructions

The Ni-CAM resin is stored in 30% ethanol. The ethanol must be removed just prior to use. Thoroughly resuspend the resin with gentle inversion and remove an appropriate aliquot for use. Take only the amount of resin that is necessary for the purification to be done. The resin may then be poured into a clean chromatography column using standard techniques or handled batch-wise for trial scale or large scale preparations. The ethanol storage solution must be removed as it may cause precipitation of some buffer salts. In general, the resin is first washed with 1-2 volumes of deionized water to remove the ethanol and then equilibrated with 3-5 volumes of equilibration buffer.

Prepare the following buffers for use in procedures for purification of native proteins. For native conditions, the equilibration buffer and the wash buffer are the same.

1. Equilibration and Wash Buffer: 50 mM sodium phosphate, pH 8.0, with 0.3 M sodium chloride and 10 mM imidazole
   A typical equilibration buffer consists of 50 mM sodium phosphate with 1 to 20 mM imidazole and 0.15 to 0.5 M sodium chloride, pH 8.0.

2. Elution Buffer: 50 mM sodium phosphate, pH 8.0, with 0.3 M sodium chloride and 250 mM imidazole

Storage/Stability
Ni-CAM affinity resin is stable for at least one year when stored properly. The Ni-CAM affinity resin should be cleaned after each use as described and an antimicrobial agent such as 20–30% ethanol should be added to the storage buffer.

Procedure

I. Extract Preparation
The protein sample may be a crude cell exact or a partially purified protein fraction prepared by standard techniques. The protein sample preparation steps should be empirically determined by the end user since the conditions may vary for different proteins.
The CelLytic B Cell Lysis Reagents (Product Nos. B7435 and B7310) supplemented with 1 to 20 mM imidazole (Product No. I 0125) are recommended for use in cell lysis. Prior to application to the resin, the protein sample must be clarified by centrifugation or filtration. For optimal results, the pH of the sample buffer must be between pH 7.0 and 8.0. The equilibration and sample buffer should be supplemented with 1 to 20 mM imidazole and 0.15 to 0.5 M sodium chloride to reduce non-specific protein binding. Consult the reagent compatibility chart for the use of other reagents.

II. Trial Scale Purification (mini-prep)
A trial scale experiment (less than 1 mg of target protein) should be performed before attempting a large-scale purification to determine if the standard operating conditions will work for the protein of interest. All steps may be performed at room temperature or at 2–8 °C.

1. Add 25-50 µl of Ni-CAM affinity resin suspension to a microcentrifuge tube and centrifuge for 30 seconds at 5,000 x *g.*
2. Carefully remove the supernatant and discard.
3. Add 200 µl of equilibration buffer and mix well.
4. Centrifuge for 30 seconds at 5,000 x *g*. Remove and discard the supernatant.
5. Add 100 µl of clarified protein solution and gently mix for 1 minute. Centrifuge the mixture as above and save the supernatant.
6. Wash the resin two times with at least 500 µl of wash buffer. Gently mix the resin for 10 seconds, then centrifuge for 30 seconds at 5000 x *g*. Save the wash buffer solutions for analysis either as a single pool or three fractions.
7. Elute the target protein with 50 µl of elution buffer. Add the buffer and mix the resin well.
8. Centrifuge for 30 seconds at 5000 x *g*.
9. Repeat steps 7 and 8 to recover more of the protein. Most of the protein will elute off in the first 50 µl fraction, but some residual protein may be eluted in the second cycle. Save the two fractions as a single pool or separate fractions.
10. Analyze all of the fractions by SDS-PAGE to determine if your target protein bound to the resin and was eluted. It is useful to perform a Western blot to determine where the histidine containing proteins are fractionated during the purification trial. If the target protein did not bind and elute from the resin, refer to the Troubleshooting guide. It may be necessary to repeat the trial under denaturing conditions.

III. Large Scale Purification
All steps may be performed at room temperature or at 2–8 °C.

A. Native Conditions - Column Chromatography

1. Transfer the appropriate amount of Ni-CAM affinity resin to a chromatography column. Wash the resin with 2 volumes of deionized water and then 3 volumes of equilibration buffer. Remove most of the equilibration buffer from the top of the column before use. Do not allow the resin to remain in equilibration buffer for extended periods of time (>24 hours) without antimicrobial agents.
2. The amount of resin required depends upon the amount of target histidine containing proteins in the extract. The target protein concentration should not exceed the capacity of the resin in the column. This should be determined for each protein to be purified.
3. Load the clarified crude extract onto the column at a flow rate of 2-10 column volumes/hour. It is recommended that the cell extract be loaded as soon as it is made and that the loading time should not exceed 6 hours. If loading time will be excessive, the protein binding may be performed using the batch format (Section III-B, steps 1-8). The resin is then placed in a column and the wash and elution are performed using steps 4 and 5, which follow immediately in this section.
4. After all of the extract is loaded, wash the column with wash buffer. The flow rate of the wash buffer should be about 10-20 column volumes/hour. The column should be extensively washed until the A_{280} of the material eluting from the column is stable and near that of the wash buffer.

5. The histidine containing protein is eluted from the column using 3-10 column volumes of elution buffer. Collect fractions and assay for the target protein. The flow rate of the elution buffer should be 2-10 column volumes/hour.

B. Native Conditions - Batch Purification Method

1. Add the appropriate amount of resin suspension to a large centrifuge tube. Centrifuge the mixture at 5,000 x *g* for 5 minutes to pellet the resin and then discard the supernatant. Alternatively, remove the supernatant by filtration.
2. Resuspend the resin in 10 resin volumes of equilibration buffer.
3. Centrifuge or filter the resin to collect it after equilibration.
4. Remove and discard the supernatant.
5. Add the cell extract to the resin. Gently mix the material on an orbital shaker (~175 rpm) for 15 minutes. Do not use a stir plate; the stir bar will break the resin beads.
6. Centrifuge the mixture at 5,000 x *g* for 5 minutes or filter. Remove and save the supernatant for SDS-PAGE analysis.
7. Add 10 volumes of wash buffer to the resin.
8. Mix the resin suspension on an orbital shaker (~175 rpm) for 4 minutes. Centrifuge the suspension at 5,000 x *g* for 5 minutes or filter.
9. Repeat steps 7 and 8 to wash the resin again.
10. The resin can be washed further until the A_{280} of the eluate no longer decreases. Discard the washes.
11. Add 2 resin volumes of elution buffer. Mix the resin on an orbital shaker (~175 rpm) for 10 minutes.
12. Centrifuge the mixture at 5,000 x *g* for 5 minutes or filter. Remove and save the supernatant or filtrate. The histidine containing protein will be in this fraction.
13. Repeat steps 11 and 12 to elute more protein. Save the eluted fractions as a single pool or separate fractions.

IV. Denaturing Conditions

Ni-CAM affinity resin can be used to purify proteins under denaturing conditions. If denaturing conditions must be used, the protein must first be solubilized with 6 M guanidine hydrochloride (Product No. G3272) or 8 M urea (Product No. U1250). Make sure the pH of the denatured cell extract is between pH 7.5-8.0 before applying it to the resin. The same purification procedures employed above can be used with denaturing buffers.

Note: Any buffers that contain urea must be made fresh daily.

An example of a urea denaturing system is described below:

Equilibration Buffer: 0.1 M sodium phosphate with 8 M urea, pH 8.0

Wash Buffer: 0.1 M sodium phosphate with 8 M urea, pH 6.3

Elution Buffer: 0.1 M sodium phosphate with 8 M urea, pH 4.5-6.0

The elution buffer pH may have to be varied because some proteins will not elute in the pH 5.0-6.0 range. If the proteins will not elute in this range, try a pH as low as 4.5.

V. Cleaning Resin for Reuse

The resin should be cleaned after every run to ensure that it will function properly on the next use. If the same crude extract is used and it has been made using the CelLytic B Cell Lysis Reagents, the column can usually be regenerated with just equilibration buffer. The detergent in the CelLytic B Cell Lysis Reagent prevents most non-specific protein binding to the resin if used as directed. This has been done over 20 times with a crude *E. coli* extract with no loss of binding capacity or purity of the final product.[3]

All steps may be performed at room temperature or at 2–8 °C.

A. General Cleaning

1. Wash the resin in the column with 2 column volumes of deionized water.
2. Clean the resin with 5 column volumes of 6 M guanidine HCl (Product No. G3272), pH 7.5. The flow rate should be no more than 5 column volumes per hour.
3. Remove the guanidine HCl solution by washing with 2-3 column volumes of deionized water.
4. The resin can now be re-equilibrated with 2-3 column volumes of equilibration buffer for immediate use or it can be washed with 1-2 column volumes of 20% ethanol and then resuspended in 20% ethanol for storage at 2–8 °C.

B. Reloading of the Nickel to the Resin

If the Ni-CAM affinity resin turns from a blue to a brown or gray color, the oxidation state of the nickel has been reduced. The reduced nickel must be removed and the resin recharged using the following procedure.

1. Wash the resin in the column with 2 column volumes of deionized water.

2. Clean the resin with 5 column volumes of 6 M guanidine HCl (Product No. G3272), pH 7.5. The flow rate should be no more than 5 column volumes per hour.
3. Remove the guanidine HCl solution by washing with 2-3 column volumes of deionized water.
4. Wash the resin with 5 column volumes of 0.1 M EDTA (Product Code ED4S), pH 7.0 to 8.0.
5. Wash the resin with 2 column volumes of deionized water.
6. Recharge the column with 2 column volumes of 10 mg/ml of nickel sulfate hexahydrate (Product No. N4882).
7. Wash the resin with 2 column volumes of deionized water.
8. The resin can now be re-equilibrated with column volumes of equilibration buffer for immediate use or it can be washed with 1-2 column volumes of 20% ethanol and then resuspended in 20% ethanol for storage at 2–8 °C.

Note: The resin can also be cleaned with 0.2 M acetic acid, 1-2% SDS, or ethanol. The ethanol can be used up to 100%, but the concentration percentage must be gradually increased and decreased in increments of no more than 25% (*i.e.*, 25, 50, 75, 100, 75, 50, 25, 0) to prevent rapid volume changes of the resin.

Results

SDS-PAGE analysis of eluted proteins should give nearly single banded material under most circumstances. The Ni-CAM affinity resin should bind at least 5 mg protein per ml of resin. The capacity is dependent on the protein being purified and the conditions used for the purification. Modification of conditions may enhance the binding capacity as well as the purity of the final product. See the troubleshooting guide for more recommendations.

When running SDS-PAGE on samples that contain guanidine HCl one must first precipitate the sample with trichloroacetic acid (TCA). This can also be done to concentrate a protein sample. Add 100% TCA solution (Product No. T6099) to the protein sample to give a final TCA concentration of 10%. Incubate the sample on ice for 15 minutes and then centrifuge the sample at full speed for 15 minutes. Carefully remove the supernatant with a pipette and resuspend the pellet in sample buffer appropriate for SDS-PAGE.

References

1. Porath, J., *et al*., Metal chelate affinity chromatography, a new approach to protein fractionation, Nature, **258,** 598-599 (1975).
2. Sulkowski, E., Purification of proteins by IMAC, Trends in Biotechnology, **3**, 1-12 (1985).
3. Sigma R&D Data
4. Porath, J., and Olin, B., Immobilized metal ion affinity adsorption and immobilized metal ion affinity chromatography of biomaterials. Serum protein affinities for gel-immobilized iron and nickel ions, Biochem., **22**, 1621-1630 (1983).
5. Anderson, L., *et al*., Facile resolution of α-fetoproteins and serum albumin by immobilized metal affinity chromatography, Cancer Res., **47**, 3624-3626 (1987).

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Reagent Compatibility Chart

Reagent	Effect	Comments
Imidazole	Binds to the nickel charged resin and competes with the histidine containing proteins	No more than 20 mM is suggested in the extract, equilibration, and wash buffers to prevent non-specific binding of proteins. No more than 250 mM is suggested for the elution buffers. Many proteins will elute with imidazole levels as low as 100 to 200 mM.
Histidine	Binds to the nickel charged resin and competes with the histidine containing proteins	Can be used in place of imidazole in the extraction, equilibration, wash, and elution buffers. No more than 250 mM is suggested for the elution buffers.
Chelating agents, *e.g.* EDTA, EGTA	Strips nickel ions from the resin	Not recommended as a buffer component, because of its ability to remove nickel ions. Can be used to strip and recharge the resin with fresh metal ions.
Guanidine HCl	Solubilize proteins	Use 6 M guanidine HCl to denature proteins and for cleaning of the resin.
Urea	Solubilize proteins	Use 8 M urea for purification under denaturing conditions.
Sodium phosphate	Used in equilibration, wash, and elution buffers to help prevent non-specific binding and buffer the solution	Recommended buffer at 50 to 100 mM for purification with the resin. The pH of any buffer should be between 7-8 with the higher capacity at the higher pH.
Sodium chloride	Prevents ionic interactions	Used in equilibration, wash, and elution buffers to help prevent binding of non-specific proteins to the resin. Recommended levels are 0.15 to 0.5 M, but up to 2 M can be used.
2-Mercapto-ethanol	A reducing agent used to prevent disulfide bonds formation	Add up to 20 mM in the extract buffer to prevent disulfide bonds. Higher levels may reduce the nickel ions.
Ethanol	Antimicrobial Also eliminates hydrophobic bonds between proteins	The binding, washing, eluting, and storage buffers may contain up to 30% ethanol.
Glycerol	Can help stabilize proteins	The binding, washing, eluting, and storage buffers may contain up to 50% glycerol.
DTE, DTT	Reduces nickel ions	Not recommended.
Nonionic detergents (TRITON™ X-100, TWEEN® 20, IGEPAL® CA-630)	Helps prevent non-specific binding of proteins to the resin	Up to 2% may be used.
Glycine	Binds weakly to resin and competes weakly with histidine containing proteins	Not recommended; use histidine or imidazole instead.

Troubleshooting guide

Problem	Cause	Solution
Protein will not bind to resin	Incorrect conditions for binding	Verify the pH and composition of sample and equilibration buffers. Make sure there are no chelating or reducing agents present in the extraction buffer.
	Histidine protein is not present	Run a Western blot of the extract to verify that the target protein is present.
	The histidines are hidden within the protein structure	Run the resin under denaturing conditions.
	Cells not extracted	Make sure that the cell extract contains target protein.
Protein elutes in the wash buffer before the elution buffer is even introduced	Wash stringency is too high	Lower the concentration of imidazole and verify that the pH is about 7-8.
	The histidines are hidden within the protein structure	Make sure the wash conditions are not too stringent. Run the resin under denaturing conditions.
Protein precipitates during purification	Temperature is too low	Run the column at room temperature.
	Protein aggregates	Add stabilizing agents such as 5-10% glycerol, 0.1% TRITON X-100 or TWEEN 20. Increase the sodium chloride concentration to 2 M. Add reducing agent such as 2-mercaptoethanol up to 20 mM. Add metals or cofactors to stabilize protein.
Pressure problems with column	Extract contains insoluble material	The protein extract must be free of insoluble material before it is loaded into the column. Insoluble material may be removed by centrifugation or filtration through a 0.45 µm membrane.
Resin no longer looks like its original color	Extract exposure	During purification many protein extracts tend to discolor a resin during the loading step. The original color will return after the wash or elution step.
	Needs to be recharged	The resin was used and cleaned numerous times, so it is time to recharge the resin with nickel.
	Loses color during run and does not regain it by the end of the run	Do not use oxidizing or reducing agents in any of the buffers or extracts. Strip and recharge the resin with nickel.
Protein will not elute off the resin	Elution conditions are too mild	Increase the amount of imidazole. For a denaturing purification make sure the pH is low enough to elute the protein; adjust elution buffer to pH 4.5. Perform a batch purification so that high protein concentrations are avoided.
Non-specific proteins elute with the target histidine protein	Binding and wash conditions are not strict enough	Increase the amount of imidazole in the extract and wash buffers up to 20 mM.
	Contaminants are related to the target histidine protein	Increase the ratio of the CelLytic B Cell Lysis Reagent to 10 or 20 ml/g of cell paste.
	Target protein is being degraded by proteases	Add protease inhibitor cocktail (Product No. P8849).
	Material is linked by disulfide bonds	Add reducing agent such as 2-mercaptoethanol, up to 20 mM.