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Product Information

HIS-Select® Cartridge

Catalog Number **H8286**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The HIS-Select Cartridge is a ready to use cartridge (column) that contains 1.25 ml of HIS-Select Nickel Affinity Gel. The cartridge's Luer lock fittings and accompanying adapters are suitable for Low Pressure Liquid Chromatography Systems (LPLC), Medium Pressure Liquid Chromatography Systems (MPLC), and for use with syringes.

HIS-Select Nickel Affinity Gel is an immobilized metal-ion affinity chromatography (IMAC) product. The HIS-Select Nickel Affinity gel is a proprietary quadridentate chelate on beaded agarose charged with nickel that is designed to specifically bind histidine containing proteins. The matrix for this affinity gel is 6% beaded agarose. HIS-Select Nickel Affinity Gel is selective for recombinant proteins with histidine tags and exhibits low non-specific binding of other proteins. The selectivity can be modulated with the inclusion of imidazole during chromatography. Recombinant proteins with histidine tags are bound using either native or denaturing conditions. The capacity of this cartridge is protein dependent but is typically ≥15 mg of histidine tagged protein.

Equipment and Reagents Required but Not Provided

(Catalog Numbers are provided as appropriate.)

- Bacterial lysis buffer such as CelLytic™ B (B7435, B7310, or C8740), CelLytic B Plus Kit (CB0500), or CelLytic Express (C1990)
- Protease Inhibitor Cocktail for use in purification of histidine-tagged proteins, DMSO solution (P8849)
- Imidazole (I0125)
- Sodium chloride (S3014)
- Sodium phosphate (S0751)
- Guanidine HCl (G3272)
- Urea (U1250)
- Nickel(II) sulfate hexahydrate (N4882)
- Low or Medium Pressure Liquid Chromatography (LPMC/MPLC) System
- Syringe
- Centrifuge
- Alternative Luer lock adapters

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 cartridge to remain for extended periods of time (>24 hours) without the addition of a suitable antimicrobial agent (i.e., 30% ethanol). Do not allow the cartridge to dry out. The recommended flow rate is 1.0 ml/min. **Do not exceed 30 psi pressure while using the cartridge.**

Note: Buffers or reagents that chelate metal ions should **not** be used with this product since they may strip the metal ions from the gel matrix. 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

Note: It is recommended that the entire technical bulletin be read before use, especially the Reagent Compatibility Chart.

The HIS-Select Cartridge contains 30% ethanol. Do not let the cartridge dry out; keep both ends capped when not in use. The ethanol storage solution must be removed just prior to use as it may cause precipitation of some buffer salts. In general, the cartridge is first washed with 5–10 ml of deionized water to remove the ethanol and then equilibrated with 5–10 ml of equilibration buffer. Hose barbed Luer lock fittings have been included for low-pressure use.

Prepare the following buffers for use in procedures for purification of recombinant proteins with histidine containing tags. 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, 0.3 M sodium chloride, 10 mM imidazole
   A typical equilibration buffer consists of 50 mM sodium phosphate, 1–20 mM imidazole, and 0.15–0.5 M sodium chloride, pH 8.0.

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

Storage/Stability
The HIS-Select Cartridge should be stored at 2–8 °C and is stable for at least one year when stored properly. The HIS-Select Cartridge has a storage buffer that contains 30% ethanol. The HIS-Select Cartridge should be cleaned after each use as described in the Procedure and an antimicrobial agent such as 30% ethanol should be added to the storage buffer.

Procedure
I. Extract Preparation
The recombinant protein with a histidine containing tag may be extracted from a crude cell extract or a partially purified protein fraction prepared by standard techniques. The protein sample preparation procedure should be determined empirically by the researcher based on the nature of the recombinant protein and the host organism. CelLytic B or CelLytic Express is recommended for lysing *E. coli* cells. Prior to application to the spin column, the recombinant protein sample must be clarified by centrifugation or filtration unless CelLytic Express is used. CelLytic Express provides a one-step extraction method that eliminates the need for cell harvest or clarification of lysates prior to purification. For optimal results, the pH of the sample buffer must be between 7.0–8.0. The equilibration and sample buffers should be supplemented with 0.15–0.5 M sodium chloride to reduce non-specific protein binding. Imidazole (up to 20 mM, typically 5–10 mM is necessary) can also be added to increase the specificity for high purity final product. Consult the Reagent Compatibility Chart for the use of other reagents.

All steps may be performed at room temperature or at 2–8 °C. A flow rate of ~1.0 ml per minute is suggested for initial work.

A. Native Conditions
This procedure can be performed using a LPLC/MPLC system, a syringe, or gravity set up. At no time allow the matrix in the cartridge to dry out. If the cartridge has insufficient liquid in it, channels will form in the gel bed.
1. Wash the HIS-Select Cartridge with 5–10 ml of deionized water and then 5–10 ml of Equilibration Buffer.
2. Prepare a clarified crude extract or CelLytic Express lysate as previously described. It is recommended that the cell extract be loaded as soon as it is made and that the loading time not exceed 6 hours.
3. The capacity of the HIS-Select Cartridge should be determined for each protein to be purified.
4. Load the clarified crude extract onto the cartridge.
5. After the extract is loaded, wash the cartridge with Wash Buffer. The cartridge should be extensively washed until the A_{280} of the material eluting from the cartridge is near that of the Wash Buffer.
6. Elute the histidine containing protein from the cartridge using 5–10 ml of Elution Buffer. Collect fractions and assay for the target protein.

B. Denaturing Conditions
HIS-Select Nickel Affinity Gel can be used to purify proteins under denaturing conditions. If denaturing conditions are to be used, the protein must first be solubilized with 6 M guanidine hydrochloride or 8 M urea. Adjust the denatured cell extract to between pH 7.0–8.0 before applying it to the cartridge. The same purification procedures employed above can be used with denaturing buffers.
Note: Any buffers that contain urea must be prepared fresh daily. An example of a urea denaturing system is described as follows:

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

Wash Buffer:
0.1 M sodium phosphate, pH 8.0, 8 M urea

Elution Buffer:
0.1 M sodium phosphate, pH 4.5 to 6.0, 8 M urea;
or
0.1 M sodium phosphate pH 8.0, 8 M urea, 250 mM imidazole

The pH of the elution buffer should be varied for histidine-tagged recombinant proteins that will not elute in the pH 5.0–6.0 range. If the tagged proteins will not elute in this range, try a pH as low as 4.5.

II. Cleaning HIS-Select Cartridge for Reuse

The HIS-Select Cartridge should be cleaned after every run. If the same crude extract is to be used and it has been made using CelLytic B or CelLytic Express, the cartridge can usually be regenerated with just Equilibration Buffer. The detergent in CelLytic B prevents most non-specific protein binding to the cartridge if used as directed. Cleaning and reuse more than 20 times has been demonstrated with no loss of binding capacity or purity of the final product.

All steps may be performed at room temperature or at 2–8 °C with flow rate of ~1.0 ml/min.

A. General Cleaning

1. Wash the cartridge with 5–10 ml of deionized water.
2. Clean the cartridge with 5–10 ml of 6 M guanidine HCl (Catalog Number G3272), pH 7.5.
3. Remove the guanidine HCl solution by washing with 5–10 ml of deionized water.
4. For immediate use, re-equilibrate the gel with 5–10 ml of equilibration buffer. If the cartridge is to be stored, wash with 5–10 ml of 30% ethanol and store at 2–8 °C. The top and bottom caps of the cartridge must be secured to prevent leakage.

B. Recharging HIS-Select Cartridge

If the HIS-Select Nickel Affinity Gel inside the cartridge turns from a blue to a brown or gray color, the nickel has been reduced. The reduced nickel must be removed and the affinity gel recharged with nickel using the following procedure:

1. Wash the cartridge with 5–10 ml of deionized water. Use a flow rate of ~1.0 ml/min for this procedure.
2. Clean the cartridge with 5–10 ml of 6 M guanidine HCl, pH 7.5.
3. Remove the guanidine HCl solution by washing with 5–10 ml of deionized water.
4. Wash the cartridge with 5–10 ml of 0.1 M EDTA, pH 7.0–8.0.
5. Wash the cartridge with 5–10 ml of deionized water.
6. Recharge the cartridge with 5–10 ml of a 10 mg/ml solution of nickel sulfate.
7. Wash the cartridge with 5–10 ml of deionized water.
8. The cartridge can now be equilibrated with 5–10 ml of Equilibration Buffer for immediate use or washed with 5–10 ml of 30% ethanol and stored at 2–8 °C. Make sure the top and bottom of the cartridge are capped for storage.

   Note: The cartridge can also be cleaned with 0.2 M acetic acid, 1–2% SDS, or ethanol. The ethanol concentration can be as high as 100%, but the ethanol concentration must be gradually increased or decreased in stages. Each stage should have an increase in ethanol concentration of no more than 25% (v/v) (i.e., 25, 50, 75, 100, 75, 50, 25, 0) to prevent rapid volume changes of the affinity gel in the cartridge.

Results

Several different recombinant proteins purified by this procedure each showed essentially a single band when assayed by SDS-PAGE. The gel matrix bound ~15 mg of protein. Binding capacity is dependent on the nature and size of the tagged recombinant 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 additional recommendations.

Before running SDS-PAGE on samples containing guanidine HCl, precipitate the protein with TCA using the ProteoPrep™ Protein Precipitation Kit, Catalog Number PROTPR, or a similar procedure.

Reagent Compatibility Chart

Reagent	Effect	Comments
Imidazole	Competes with histidine-tagged recombinant proteins for binding sites on nickel-charged affinity gel	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 100–200 mM.
Histidine	Binds to the nickel charged affinity gel and competes with the histidine-tagged proteins	May be used in place of imidazole in the extraction, equilibration, wash, and elution buffers.
Chelating agents (EDTA, EGTA)	Strip nickel ions from the affinity gel	Not recommended as buffer components because they can remove nickel ions. Used to strip the affinity gel of metal before recharging with fresh metal ions.
Guanidine HCl	Solubilizes proteins	Use 6.0 M to denature proteins and cleaning the cartridge.
Urea	Solubilizes 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 to buffer the solution	Recommended buffer at 50–100 mM for purification with the affinity gel. The pH of any buffer should be between 7 and 8 with higher binding 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 affinity gel. Recommended levels are 0.15–0.5 M, but up to 2.0 M may be used.
2-Mercaptoethanol	A reducing agent used to prevent disulfide bond formation	Add up to 20 mM in the extract buffer to break disulfide bonds. Higher levels may reduce the nickel ions.
DTE, DTT	Reduce nickel ions	Not recommended.
Ethanol	Acts as an antimicrobial agent; eliminates hydrophobic interaction between proteins	The binding, washing, eluting, and storage buffers may contain up to 30% ethanol.
Glycerol	May help stabilize proteins	The binding, washing, eluting, and storage buffers may contain up to 50% glycerol.
Nonionic detergents (TRITON®, TWEEN®, IGEPAL®CA-630)	Help prevent non-specific binding of proteins to the affinity gel	Up to 2% may be used.
Glycine	Binds weakly to affinity gel and competes weakly with histidine containing proteins	Not recommended; use histidine or imidazole instead.

Troubleshooting Guide

Problem	Cause	Solution
Recombinant protein with histidine tag will not bind to affinity gel	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.
	Recombinant protein is not present.	Run a Western blot of the extract to verify the recombinant protein is present.
	The histidine tag is buried within the protein structure.	Run the affinity purification under denaturing conditions.
	Cells not extracted	Make sure the cell extract contains target protein.
Protein elutes in the wash buffer before the elution buffer is even introduced	Wash stringency is too high.	Make sure the wash conditions are not too stringent. Lower the imidazole concentration and verify that the pH is between 7 and 8. Under denaturing conditions without imidazole raise the pH to 8.
	The histidine tag is buried within the protein structure.	Make sure the wash conditions are not too stringent. Run the affinity purification under denaturing conditions.
Protein precipitates during purification	Temperature is too low	Run the procedure at room temperature.
	Protein aggregates	Add stabilizing agents such as 5–50% glycerol, 0.1% TRITON X-100 or TWEEN 20. Increase the sodium chloride concentration up to 2 M. Add reducing agent such as 2-mercaptoethanol up to 20 mM. Add metals or cofactors to stabilize protein.
Pressure problems with cartridge	Extract contains insoluble material.	The protein extract must be free of insoluble material before it is loaded into the cartridge. Insoluble material may be removed by centrifugation or by filtration through a 0.45 μm membrane.
Affinity gel changes color	Extract exposure	During purification many protein extracts tend to discolor an affinity gel during the loading step. The original color will return after the wash or elution step.
	Loss of nickel	Recharge the affinity gel 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 affinity gel with nickel.
Recombinant protein with histidine tag will not elute from the affinity gel	Elution conditions are too mild.	Increase the amount of imidazole. For a denaturing purification make sure the pH is low enough to elute the tagged recombinant protein; adjust elution buffer to pH 4.5. Perform batch purification so that high protein concentrations are avoided.
The top of the cartridge (female Luer lock end) popped off	Excessive pressure was applied.	**Do NOT exceed 1.0 ml/min or 30 psi with this cartridge.**
Channels are present within the gel bed	The cartridge was allowed to dry out.	Run suitable buffer back and forth through both ends of the cartridge using two syringes until the channels diminish.
	Air entered the cartridge.	Degas the buffers that will be used in the protein purification process.
Non-specific proteins elute with the histidine tagged recombinant protein	Binding and wash conditions are not strict enough.	Increase the amount of imidazole in the extract and wash buffers up to 20 mM.
	Target protein is being degraded by proteases.	Add Protease Inhibitor Cocktail (Catalog Number P8340).