Product Information

HIS-Select® HF Nickel Affinity Gel

H0537
Storage Temperature 2–8 °C

Product Description

HIS-Select® High Flow (HF) is an immobilized metal-ion affinity chromatography (IMAC) product that consists of a proprietary quadridentate chelate on highly cross-linked beaded agarose charged with nickel.

HIS-Select® HF is designed to specifically bind histidine-containing proteins in chromatographic systems with pressures up to 200 psi or a maximum linear flow rate of 3,000 cm/hr.

HIS-Select® HF is durable and can capture recombinant proteins with histidine tags at high flow rates while exhibiting low non-specific binding of other proteins. The selectivity of the affinity resin can be modulated with imidazole, a histidine analog, during capture and washing steps. Recombinant proteins with histidine tags can be captured under native or denaturing conditions. The capacity of this affinity gel is typically about 10–20 mg histidine-tagged protein per mL of packed gel. The matrix for this affinity gel is 6% highly cross-linked beaded agarose.

Reagent

HIS-Select® HF is supplied as a 50% suspension in 30% ethanol.

Reagents and Equipment Required (Not included)

(Cat. No. have been suggested where appropriate)

- Appropriate centrifuge
- CelLytic™ Cell Lysis and Protein Extraction Reagent (Formulations are available for various cell types)
- Appropriate column or centrifuge tubes
- Imidazole (Cat. No. I5513)
- Sodium chloride (Cat. No. S3014)
- Sodium phosphate (Cat. No. S0751)
- Protease Inhibitor Cocktail (Cat. No. P8340, P8849, S8830, MSSAFE)

It is recommended that the entire Technical Bulletin be read before use, especially the Reagent Compatibility Chart.

Precautions and Disclaimer

For research use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Do not allow the affinity gel to remain in any buffer for extended periods of time (> 24 hours) unless an antimicrobial agent (for example, 30% ethanol) is added to the buffer.

Note: Buffers or reagents that chelate metal ions should **not** be used with this product, since they may strip the metal ions 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 the Reagent Compatibility Chart for more information.

Preparation Instructions

The HIS-Select® HF is stored in 30% ethanol. The ethanol must be removed just prior to use. Thoroughly resuspend the affinity gel with gentle inversion and remove an appropriate aliquot for use. The affinity gel 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 affinity gel 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 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, with 0.3 M sodium chloride and 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, with 0.3 M sodium chloride and 250 mM imidazole.

Storage/Stability

HIS-Select® HF is stable for at least one year when stored at 2-8 °C. The HIS-Select® HF should be cleaned after each use (see Procedure, General Cleaning) and an antimicrobial agent such as 30% ethanol should be added to the storage buffer.

Procedure

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 end user should empirically determine the protein sample preparation steps since the conditions may vary depending on the nature of the recombinant protein and the host organism. CelLytic™ products are available for various expression systems and are recommended for use with the HIS-Select® HF Gel. Prior to application to the affinity gel, the recombinant 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 1-20 mM imidazole and 0.15-0.5 M NaCl to reduce non-specific protein binding. Consult the Reagent Compatibility Chart for the use of other reagents.

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 recombinant protein of interest.

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

1. Add 25-50 µL of HIS-Select® HF suspension to a microcentrifuge tube and centrifuge for 30 seconds at 5,000 × *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 × *g*. Remove and discard the supernatant.
5. Add 100 µL of clarified recombinant protein solution and gently mix for 1 minute. Centrifuge the mixture for 30 seconds at 5,000 × *g* and save the supernatant.
6. Wash the affinity gel with at least 500 µL of Wash Buffer. Gently mix the affinity gel for 10 seconds, then centrifuge for 30 seconds at 5,000 × *g*. Save the Wash Buffer solutions for analysis either as a single pool or as three fractions. Repeat the wash step one more time.
7. Elute the target protein with 50 µL of Elution Buffer. Add the buffer and mix the affinity gel well.
8. Centrifuge for 30 seconds at 5,000 × *g*. Carefully remove the supernatant and save for analysis.
9. Repeat steps 7 and 8 to recover more of the protein. Most of the protein will be eluted 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 as separate fractions.
10. Analyze all the fractions by SDS-PAGE to determine if your target protein bound to the affinity gel and was eluted. It is useful to perform a Western blot to determine which fractions contain the histidine-tagged proteins. If the target protein did **not** bind and elute from the affinity gel, refer to the Troubleshooting Guide. It may be necessary to repeat the trial under denaturing conditions.

Large-Scale Purification

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

Native Conditions - Column Chromatography

1. Transfer the appropriate amount of HIS-Select® HF to a chromatography column. Wash the affinity gel with 2 volumes of deionized water to remove ethanol and then 3 volumes of Equilibration Buffer. Remove most of the Equilibration Buffer from the top of the column before use.
   Note: Do not allow the affinity gel to remain in Equilibration Buffer for extended periods of time (> 24 hours) without antimicrobial agents.
2. The amount of affinity gel required depends upon the amount of target histidine-containing proteins in the extract. The target protein concentration should not exceed the capacity of the affinity gel in the column. The amount of resin needed 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. If loading time will be excessive, the protein binding may be performed using the batch format (Native Conditions – Batch Purification Method, Steps 1-8) or protease inhibitors may be added. The batch loaded affinity gel 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 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 should be 2–10 column volumes/hour.

Native Conditions - Batch Purification Method

1. Add the appropriate amount of affinity gel suspension to a large centrifuge tube. Centrifuge the mixture at 5,000 × *g* for 5 minutes to pellet the affinity gel and then discard the supernatant. Alternatively, remove the supernatant by filtration.
2. Resuspend the affinity gel in 10 gel volumes of Equilibration Buffer.
3. Centrifuge or filter the affinity gel to collect it after equilibration.
4. Remove and discard the supernatant.
5. Add the clarified cell extract to the affinity gel. Gently mix the material on an orbital shaker for 15 minutes. Do not use a stir plate, as the stir bar will break the affinity gel beads.
6. Centrifuge the mixture at 5,000 × *g* for 5 minutes or filter. Remove and save the supernatant for SDS-PAGE analysis.
7. Add 10 volumes of Wash Buffer to the affinity gel.
8. Gently mix the affinity gel suspension on an orbital shaker for 4 minutes. Centrifuge the suspension at 5,000 × *g* for 5 minutes or filter.
9. Repeat steps 7 and 8 to wash the affinity gel again.
10. The affinity gel can be washed further until the A_{280} of the eluate no longer decreases. Discard the washes.
11. Add 2 gel volumes of Elution Buffer. Gently mix the affinity gel on an orbital shaker for 10 minutes.
12. Centrifuge the mixture at 5,000 × *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 as separate fractions.

Denaturing Conditions

HIS-Select® HF 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 (Cat. No. G3272) or 8 M urea (Cat. No. U1250). Make sure the pH of the denatured cell extract is between pH 7.0 to 8.0 before applying it to the affinity gel. The same purification procedures employed for nondenaturing conditions can be used with denaturing buffers.
Note: Any buffers that contain urea must be made fresh daily.

An example of a urea denaturing system follows:

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

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

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

The Elution Buffer pH may have to be varied because some recombinant proteins with histidine containing tags will not elute in the pH range of 5.0-6.0. If the tagged recombinant proteins will not elute in this range, try a pH as low as 4.5, or the addition of 250 mM imidazole.

Cleaning HIS-Select® HF for Reuse

The affinity gel 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 CelLytic™ B, the column can usually be regenerated with just Equilibration Buffer. The detergent in CelLytic™ B prevents most non-specific protein binding to the affinity gel if used as directed.

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

General Cleaning

1. Wash the affinity gel with 2 column volumes of deionized water.
2. Clean the affinity gel with 5 column volumes of 6 M guanidine HCl, pH 7.5.
3. Remove the guanidine HCl solution by washing with 2-3 column volumes of deionized water.
4. The affinity gel can now be re-equilibrated with 2-3 column volumes of Equilibration Buffer for immediate use.
5. Alternatively, the affinity gel can be washed with 1-2 column volumes of 30% ethanol, and then resuspended in 30% ethanol for storage at 2-8 °C.

Note: The affinity gel 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% (for example, 25%, 50%, 75%, 100%, 75%, 50%, 25%, 0%), to prevent rapid volume changes of the affinity gel.

Recharging HIS-Select® HF

If the HIS-Select® HF 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, using the following procedure.

1. Wash the affinity gel with 2 column volumes of deionized water.
2. Clean the affinity gel with 5 column volumes of 6 M guanidine HCl, pH 7.5.
3. Remove the guanidine HCl solution by washing with 2–3 column volumes of deionized water.
4. Wash the affinity gel with 5 column volumes of 0.1 M EDTA, pH 7.0-8.0.
5. Wash the affinity gel with 2 column volumes of deionized water.
6. Recharge the column with 2 column volumes of 10 mg/mL of nickel(II) sulfate hexahydrate (Cat. No. N4882).
7. Wash the affinity gel with 2 column volumes of deionized water.
8. The affinity gel can now be re-equilibrated with 2-3 column volumes of Equilibration Buffer for immediate use.
9. Alternatively, the affinity gel can be washed with 1-2 column volumes of 30% ethanol, and then resuspended in 30% ethanol for storage at 2-8 °C.

Results

SDS-PAGE analysis of eluted recombinant proteins should give nearly single-banded material under most circumstances. The affinity gel should bind at least 15 mg of protein per mL of gel. The capacity is dependent on the nature and size of the tagged recombinant 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, a protein precipitation kit (Cat. No 539180) may be of use.

Several publications cite use of Cat. No. H0537 in their protocols.[1-12]

References

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Reagent Compatibility Chart

Reagent	Effect	Comments
Imidazole	Binds to the nickel-charged affinity gel and competes with the histidine-tagged recombinant proteins	For column chromatography, 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. For batch methods, the imidazole concentration may have to be reduced or eliminated.
Histidine	Binds to the nickel charged affinity gel 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, for example, EDTA and EGTA	Strips nickel ions from the affinity gel	Not recommended as a buffer component, because of its ability to remove nickel ions. Can be used to strip and recharge the affinity gel with fresh metal ions.
Guanidine HCl	Solubilizes proteins	Used to denature proteins and clean the affinity gel.
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 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 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 affinity gel. Recommended levels are 0.15-0.5 M, but up to 2 M can be used.
2-Mercaptoethanol	A reducing agent used to prevent disulfide bonds formation	Add up to 20 mM in the extract buffer to break 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 and DTT	Reduces nickel ions	Not recommended.
Nonionic detergents (TRITON®, TWEEN®, IGEPAL® CA-630)	Helps 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-containing 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. If using batch mode, remove imidazole.
	Recombinant protein is not present.	Run a Western blot of the extract to verify that the recombinant protein is present.
	The histidine-containing tag is buried within the protein structure	Run the affinity purification 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 7-8.
	The histidine-containing 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 column at room temperature.
	Protein aggregates	Add stabilizing agents such as 5-10% glycerol, 0.1% TWEEN® 20, or 0.1% TRITON® X-100. Increase the NaCl 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 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.
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.
	Needs to be recharged	The affinity gel was used and cleaned numerous times, so it is time to 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-containing tag will not elute off 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.
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.
	Material is linked by disulfide bonds	Add reducing agent such as 2-mercaptoethanol, up to 20 mM.

Notice

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