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Product Information

HIS-Select® High Flow Cartridge, 1.25 ml

Catalog Number **H7788**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The HIS-Select® High Flow Cartridge, 1.25 ml, is a ready-to-use cartridge (column) that contains 1.25 ml of HIS-Select High Flow (HF) Nickel Affinity Gel (Catalog Number H0537). The cartridge is suitable for Low Pressure Liquid Chromatography Systems (LPLC), Medium Pressure Liquid Chromatography Systems (MPLC), Fast Pressure Liquid Chromatography Systems (FPLC™), and for use with syringes. The cartridge has Luer lock fittings, which facilitate connection to most liquid chromatography systems when coupled to suitable adapters.

The HIS-Select HF Nickel Affinity Gel is designed for the purification of histidine-tagged proteins. The HF Nickel Affinity Gel brings the superior selectivity of HIS-Select technology to a highly cross-linked agarose for higher flow rates and mechanical stability under pressure. The medium for the affinity gel is a highly cross-linked 6% beaded agarose. As with other HIS-Select products, the non-charged, hydrophilic linkage of the nickel-nitrilotriacetic acid (Ni-NTA) chelate group to the agarose ensures highly specific purification of histidine-tagged proteins. Recombinant proteins with histidine-containing tags may be bound using either native or denaturing conditions. The capacity of this cartridge is typically at least 19 mg of histidine-tagged protein per 1.25 ml cartridge (15 mg of protein per ml of affinity gel).

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

Components

The HIS-Select High Flow Cartridge, 1.25 ml, is supplied with the packed affinity gel in a storage buffer containing 30% ethanol.

The cartridges are supplied with adapters compatible with chromatography systems, such as Amersham Biosciences' ÄKTA® system.

Reagents and Equipment Required but Not Provided

(Catalog Numbers have been given where appropriate)

- Centrifuge
- CelLytic™ Cell Lysis and Protein Extraction Reagent (formulations are available for various cell types)
- HIS-Select Wash Buffer (10 mM imidazole in 0.3 M sodium chloride and 50 mM sodium phosphate, pH 8.0, Catalog Number H5288)
- HIS-Select Elution Buffer (250 mM imidazole in 0.3 M sodium chloride and 50 mM sodium phosphate, pH 8.0, Catalog Number H5413)
- 0.5 M EDTA Solution (Catalog Number E7889)
- Nickel(II) sulfate, hexahydrate (Catalog Number N4882)
- Fast Pressure Liquid Chromatography System (FPLC)
- Syringe (optional)
- Guanidine HCl (Catalog Number G3272)
- Urea (Catalog Number U1250)
- 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) in buffer without the presence of a suitable antimicrobial agent (e.g., 30% ethanol). Do not allow the cartridge to dry out.

The recommended flow rate is 1 ml/min or less. **Do not exceed a pressure of 100 psi while using the cartridge.** 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 ions and thus inhibit the binding of histidine-containing proteins. See the Reagent Compatibility Chart for more information.

Preparation Instructions
The HIS-Select High Flow Cartridge contains a 30% ethanol storage buffer. Do not allow the cartridge to dry out; keep both ends tightly capped when not in use. The 30% ethanol storage buffer must be removed just prior to use, as it may cause precipitation of some buffer salts. Wash the cartridge with 5–10 ml of deionized water to remove the ethanol solution and then equilibrate with 5–10 ml of HIS-Select Wash Buffer (10 mM imidazole in 0.3 M sodium chloride and 50 mM sodium phosphate, pH 8.0, Catalog Number H5288).

The M6 Female-Male and Female Tefzel® Luer lock adapters (Catalog Number L6667) have been included to connect the HIS-Select High Flow Cartridge to ÄKTA Fast Pressure Liquid Chromatography Systems. The Luer lock ends of the fittings connect to the HIS-Select High Flow Cartridge. If the adapters do not fit the make and model of the FPLC system used, please contact the instrument supplier to determine the correct fittings to connect this cartridge to the instrument.

Storage/Stability
The HIS-Select High Flow Cartridge is stable for at least one year when stored unopened at 2–8 °C. The HIS-Select High Flow Cartridge should be cleaned after use as described and an antimicrobial agent, such as a 30% ethanol solution, added for storage. Do not store the cartridge for extended periods of time (>24 hours) without the presence of an antimicrobial agent. Do not allow the cartridge to dry out.

If air bubbles form at one end of the cartridge, displace them by flushing the column with a 30% ethanol solution. This may be accomplished using a syringe filled with the solution. Place the column upright with the air bubbles at the top of the column and attach the syringe to the opposite end. Then gently push the ethanol solution through the column to remove the bubbles.

Procedures
I. Extract Preparation
Recombinant protein(s) with histidine-containing tags may be purified from a crude cell extract or a partially purified protein fraction prepared by standard techniques. The researcher should determine the protein sample preparation procedure empirically, because conditions depend on the nature of the recombinant protein and host organism. CelLytic products are available for various expression systems and are recommended for use with the HIS-Select High Flow Cartridge.

The lysate must be clarified by centrifugation or filtration prior to application to the cartridge. For optimal results, the pH of the cell extract should be between 7.0 and 8.0. Consult the Reagent Compatibility Chart for the use of other reagents.

II. Purification of the Target Protein
All steps may be performed at room temperature or at 2–8 °C. A flow rate of less than 1 ml/min (~50 column volumes an hour) is suggested for initial work.

A. Native Conditions
This procedure can be performed using an FPLC system, a syringe, or gravity flow set-up. Do not allow the matrix in the cartridge to dry out. If the cartridge has insufficient liquid present, channels will form in the gel bed. The capacity of the HIS-Select High Flow Cartridge should be determined empirically for each target protein to be purified.

1. Wash the HIS-Select High Flow Cartridge with 5–10 ml of deionized water to remove the storage solution, and equilibrate the cartridge with 5–10 ml of HIS-Select Wash Buffer (10 mM imidazole in 0.3 M sodium chloride and 50 mM sodium phosphate, pH 8.0, Catalog Number H5288); see Preparation Instructions.

2. Prepare a clarified crude extract as described in Procedures, Extract Preparation. It is recommended to load the cell extract as soon as it is prepared. If this is not possible, the time between preparation of the extract and application to the cartridge should not exceed 6 hours.

3. Apply the clarified crude extract to the cartridge. The recommended flow rate is 1 ml/min or less. Do not exceed a pressure of 100 psi while using this cartridge.

4. After the extract is applied, wash the cartridge with approximately 10 ml of HIS-Select Wash Buffer to remove unbound proteins. The cartridge should be washed until the A_{280} of the effluent from the cartridge is approximately that of the wash buffer.

5. Elute the histidine-tagged protein from the cartridge using 5–10 ml of HIS-Select Elution Buffer, (250 mM imidazole in 0.3 M sodium chloride and 50 mM sodium phosphate, pH 8.0, Catalog Number H5413). Collect elution fractions and assay for the target protein.

B. Denaturing Conditions

The HIS-Select High Flow Cartridge, 1.25 ml, can be used to purify proteins under denaturing conditions. If denaturing conditions are to be used, the protein must first be solubilized with a 6 M guanidine hydrochloride solution or an 8 M urea solution. Adjust the pH of the denatured cell extract to between 7.0 and 8.0 before applying to the cartridge. The same purification scheme employed for Native Conditions can be used for Denaturing Conditions with the substitution of a denaturing Wash Buffer and a denaturing Elution Buffer in steps 4 and 5, respectively.

The following is an example of a urea denaturing buffer system:

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

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

or

0.1 M sodium phosphate, pH 8.0, with 8 M urea and 250 mM imidazole

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

The pH of the elution buffer may have to be adjusted because some recombinant proteins with histidine tags will not elute in the pH range of 5.0–6.0. If the tagged recombinant protein(s) will not elute in this range, elution with a pH as low as 4.5 is suggested.

III. Care of the HIS-Select High Flow Cartridge after Use

The HIS-Select High Flow Cartridge should be cleaned after every run. When used and cleaned properly, the cartridge can be reused up to 5 times with no loss of binding capacity, or yield and purity of the final product. All steps may be performed at room temperature or at 2–8 °C with a flow rate of ~1 ml/min or less.

For consecutive samples prepared in a similar manner using a CelLytic B Reagent, the cartridge can usually be cleaned with 5–10 ml of HIS-Select Wash Buffer between runs. If used as directed, the detergent in CelLytic B prevents most proteins from non-specifically binding to the cartridge. For samples prepared without a CelLytic B Reagent, follow the General Cleaning Procedure.

A. General Cleaning Procedure

1. Wash the cartridge with 5–10 ml of deionized water. To remove bound residual proteins, clean the affinity gel cartridge with 5 ml of a 6 M guanidine HCl (Catalog Number G3272) solution.

2. Remove the guanidine HCl solution by washing with 5–10 ml of deionized water. Inspect the HIS-Select HF Affinity Gel inside the cartridge for any change from the blue color. If the blue color has disappeared or changed to brown or gray, the affinity gel needs to be recharged with fresh Ni^{2+} ions. Proceed to Care of the HIS-Select High Flow Cartridge after Use, Recharging the HIS-Select High Flow Cartridge, step 1. If there has been no color change, proceed to the next step.

3. For immediate use, re-equilibrate the gel with 5–10 ml of HIS-Select Wash Buffer. If the cartridge is to be stored, wash with 5–10 ml of a 30% ethanol solution and store at 2–8 °C. The top and bottom caps of the cartridge must be tightly secured to prevent evaporation or leakage.

   Note: The cartridge can also be cleaned with 0.2 M acetic acid, 1-2% SDS, or ethanol. A 100% ethanol solution can be used; however, the ethanol concentration must be gradually increased or decreased in stages. In each stage the ethanol concentration should change by no more than 25% (v/v) (i.e. 25, 50, 75, 100, 75, 50, 25, 0) to prevent rapid volume changes to the affinity gel in the cartridge.

B. Recharging the HIS-Select High Flow Cartridge
If the HIS-Select HF Affinity Gel inside the cartridge changes color from blue to brown or gray, the chelated nickel ions have been reduced. The reduced nickel must be removed and the affinity gel recharged with fresh Ni^{2+} ions. The recharging procedure for the affinity gel should follow the General Cleaning Procedure, step 2.

1. Wash the cartridge with 5–10 ml of 0.1 M EDTA solution (dilute from Catalog Number E7889), pH 7.0–8.0.

2. Wash the cartridge with 5–10 ml of deionized water.

3. Recharge the cartridge with 5–10 ml of a 10 mg/ml solution of nickel sulfate, hexahydrate (Catalog Number N4882).

4. Wash the cartridge with 5–10 ml of deionized water. Continue to General Cleaning Procedure, step 3 for equilibration instructions.

Results
Several different recombinant proteins purified by this procedure showed essentially a single band when assayed by SDS-PAGE. The binding capacity was at least 15 mg of a ~30 kDa histidine-tagged protein per ml of affinity gel. Binding capacity is dependent on the nature and size of the tagged recombinant protein being purified and the conditions used for the purification. See the Troubleshooting Guide for additional recommendations.

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

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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 imidazole is suggested in the extraction, equilibration, and wash buffers to prevent the non-specific binding of proteins. No more than 250 mM is suggested for the elution buffer. Many proteins will elute with imidazole concentrations as low as 100–200 mM.
Histidine	Binds to the nickel charged affinity gel and competes with histidine-tagged proteins	Can be used in place of imidazole in the extraction, equilibration, wash, and elution buffers. A concentration of no more than 250 mM is suggested for the elution buffer.
Chelating agents (e.g., EDTA or EGTA)	Strips nickel ions from the affinity gel	Not recommended as buffer components because they remove nickel ions. Used to strip the affinity gel before recharging with fresh metal ions.
Guanidine HCl	Solubilizes proteins	Used to denature proteins and for cleaning of the affinity gel.
Urea	Solubilizes proteins	Use 8 M urea for purification under denaturing conditions.
Sodium phosphate	Used in the equilibration, wash, and elution buffers to help buffer the solution and prevent non-specific binding	Recommended buffer at a concentration of 50–100 mM for purification with the affinity gel. The pH of any buffer should be between 7 and 8 with the higher molarity used at the higher pH.
Sodium chloride	Helps prevent ionic interactions	Used in equilibration, wash, and elution buffers to help prevent the binding of non-specific proteins to the affinity gel. Recommended concentration levels are 0.15–0.5 M, but up to 2 M can be used.
2-Mercaptoethanol	A reducing agent used to prevent disulfide bond formation	Add up to 20 mM 2-mercaptoethanol in the extraction buffer to reduce disulfide bonds. Higher concentrations may reduce the nickel ions and decrease binding capacity of the affinity gel.
DTE or DTT	Prevent disulfide bond formation; may reduce nickel ions	Add up to 5 mM DTE or DTT to the extraction buffer to reduce disulfide bonds. Reduction in binding capacity is often observed when using DTT.
Ethanol	Antimicrobial; also helps prevent hydrophobic interactions between proteins	The binding, washing, elution, and storage buffers may contain up to 30% ethanol. Note: Ethanol may cause the precipitation of some buffer salts. Buffers should be prepared and checked for salt precipitation before use.
Glycerol	Can help stabilize proteins	The binding, washing, elution, and storage buffers may contain up to 50% glycerol.
Nonionic detergents (e.g., TRITON® X-100, TWEEN® 20, or IGEPAL® CA-630)	Help prevent non-specific binding of proteins to the affinity gel	A concentration of up to 2% may be used.
Glycine	Binds weakly to the affinity gel, and competes weakly with histidine containing proteins	Not recommended for elution; 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 of both the sample and wash buffer is between 7 and 8. Ensure the imidazole concentration in the wash buffer is between 0–10 mM. 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 that the recombinant protein is present.
	The histidine tag is buried within the protein structure.	Run the affinity purification under denaturing conditions.
	Protein not extracted from cells.	Try different cell lysis/extraction conditions. Determine if the protein of interest is contained within insoluble inclusion bodies.
Protein elutes in the wash buffer before the elution buffer is introduced.	Wash stringency is too high.	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.	Verify that 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% TRITON X-100, or TWEEN 20. Increase the sodium chloride concentration to as much as 2 M. Add low levels of a suitable reducing agent, such as 2-mercaptoethanol - up to a 20 mM concentration. Add cofactors to sample to stabilize proteins.
Pressure problems with column	Extract contains insoluble material.	The protein extract must be free of insoluble material before it is applied to the column. Insoluble material may be removed by centrifugation or filtration through a 0.45 μm membrane.
Affinity gel (light blue) in cartridge changes color.	Exposure to extract	During purification, many protein extracts tend to discolor the affinity gel following application. The original light blue color should return after the wash or elution step.
	Loss of nickel (loss of light blue color)	Strip and recharge the affinity gel with nickel sulfate hexahydrate, (Catalog Number N4882).
	Affinity gel color becomes brown or gray due to chemical reduction of Ni^{2+} ions.	Do not use strong oxidizing or reducing agents in any of the buffers or extracts. Reduced nickel ions may irreversibly bind to the HIS-Select gel and thus often cannot be stripped. Use a new cartridge if the blue color is not restored after recharging with nickel sulfate hexahydrate.
Recombinant protein with histidine tag will not elute from the affinity gel.	Elution conditions are too mild.	Increase the concentration of imidazole in the elution buffer. For a denaturing purification with pH elution, make sure the pH is low enough to elute the tagged recombinant protein; adjust elution buffer to pH 4.5.

Troubleshooting Guide (Continued)

Problem	Cause	Solution
Non-specific proteins elute with the histidine-tagged recombinant protein.	Binding and wash conditions are not sufficiently stringent.	Increase the concentration of imidazole in the extraction and wash buffers to ≤20 mM.
	Target protein is being degraded by proteases.	Add Protease Inhibitor Cocktail (Catalog Number P8849).
	Material is linked by disulfide bonds.	Add a reducing agent such as 2-mercaptoethanol, up to a concentration of 20 mM.
The top of the cartridge (female Luer lock end) popped off.	Excessive pressure was applied.	**Do NOT exceed 1.0 ml/min or a pressure of 100 psi with this cartridge.** Run the column at lower flow rate with less pressure.
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 used in the protein purification process.