



FAQs for HIS-Select Products

1. What is the expected purity level (%) with HIS-Select?

This depends on the protein size and characteristics. Typically results show >95% purity with a 30 kDa protein.

2. Why should no more than 10 mM be used in column wash steps?

While imidazole in other resins is used to regulate non-specific binding, it will also reduce the protein recovery yield. HIS-Select's unique chemistry has been developed to minimize non-specific binding, so additional imidazole is not necessary and will reduce yield.

3. Is this resin compatible with urea?

The HIS-Select resin is composed of the same backbone as other compatible resins and therefore will have similar chemical and physical properties. The use of 8 M urea or 6 M guanidine-HCl is acceptable. You can try to do a slow gradient to lower the concentrations of urea while the protein is bound to the column. After you remove the urea via the reverse gradient method, you can elute with imidazole.

4. Are protease inhibitors necessary for protein isolation?

No. Protease inhibitors in the cell extract are not necessary, but may help prevent proteolytic degradation, thereby preserving protein activity.

5. Is this resin suitable for use with copper-containing (or other metal-containing) proteins?

If the protein already contains bound copper there should be no issues since the copper and nickel should not exchange. However, if the copper-containing protein does not have metal in it there is a slight possibility that it could remove the nickel from the resin. NTA binds nickel very tightly, however, it can be removed with 50 to 100 mM EDTA.

6. Is imidazole necessary for purification under denaturing conditions?

For the denaturing conditions it is not necessary to include the imidazole since the lower pH of 4.5 will elute the proteins under denaturing conditions. Addition of imidazole will add more salts, resulting in protein precipitation.

7. Is this resin compatible with NDSBs (non-denaturing sulfobetaines)?

NDSBs are "detergents." Experiments with selected NDSBs have found no effect on protein binding at the concentrations between 1-4%. Micelles at higher concentrations may prevent protein binding.

8. What is meant by one-step purification? There seems to be more than one step in the protein purification process.

One-step refers to the number of times the purification process needs to be performed to get a high level of purity. Many other resins require the process be performed two or more times to get high purity levels due to high non-specific binding.

9. My secreted protein won't bind.

Most media has a high iron content. Iron may displace the nickel from the resin thereby increasing the number of potential non-specific proteins binding to the column. This free metal can be removed from the media by using a small column containing the metal-free version of our IMAC resin (Product Code I 1408).



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 10 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. 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-tagged recombinant 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 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 for cleaning of 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 solutions	Recommended buffer at 50 to 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 to 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 Eliminates hydrophobic bonds between proteins	The binding, washing, eluting and storage buffers may contain up to 20% ethanol.
Glycerol	Helps stabilize proteins	The binding, washing, eluting and storage buffers may contain up to 50% glycerol.
DTE and DTT	Reduces protein disulfide bonds	Up to 5 mM DTT or DTE can be used. Higher amounts may reduce the nickel in the resin.
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-tagged recombinant 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 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 about 7 to 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 to 10% glycerol, 0.1% TRITON X-100 or TWEEN 20. Increase the sodium chloride concentration up to 2 M. Add reducing agent (2-mercaptoethanol up to 20 mM or DTT up to 5 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 mm 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 high concentrations of reducing agents in any of the buffers or extracts. Strip and recharge the affinity gel with nickel. Reduced metal may not be removed from the resin.
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 recombinant protein with the histidine-containing tag	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 (Product Code P 8340).
	Material is linked by disulfide bonds	Add reducing agent (2-mercaptoethanol up to 20 mM or DTT up to 5 mM).