

Technical Bulletin

## HIS-Select® Spin Columns

**H7787**

### Product Description

The HIS-Select® Spin Column is an immobilized metal-ion affinity chromatography (IMAC) product that allows rapid purification of small-scale crude cell extracts that contain histidine-tagged proteins. The HIS-Select® Spin Column contains 20 µm, spherical silica particles (100 nm pore size) with a hydrophilic layer. The silica is derivatized with a proprietary quadridentate chelate that is charged with nickel.

HIS-Select® Spin Columns are selective for recombinant proteins with His-tags and exhibit very low non-specific binding of other proteins. The selectivity can be modulated with the inclusion of imidazole during chromatography. The binding capacity of a HIS-Select® Spin Column is >500 µg per column, as determined with an ~30 kDa His-tagged protein that has been recovered under high levels of protein expression.

Several publications,<sup>1-6</sup> theses,<sup>7-9</sup> and dissertations<sup>10-14</sup> have cited use of product H7787 in their research protocols.

### Components

**H7787-10EA:** 10 HIS-Select® Spin Columns / Collection Tube sets with 20 additional Collection Tubes (total of 30 Collection Tubes)

**H7787-50EA:** 50 HIS-Select® Spin Columns / Collection Tube sets with 100 additional Collection Tubes (total of 150 Collection Tubes)

### Equipment Required but Not Provided

Suggested Cat. Nos. are provided as appropriate.

- Microcentrifuge or vacuum manifold
- Bacterial lysis buffer, such as CellLytic™ B (Cat. Nos. B7435, B7310, or C8740), CellLytic™ B Plus Kit (Cat. No. CB0500), or CellLytic™ Express (Cat. No. C1990)
- Imidazole, such as Cat. No. I5513
- Sodium chloride, such as Cat. No. S3014

- Sodium phosphate, such as Cat. No. S3139 (monobasic) and Cat. No. S3264 (dibasic)
- Bradford Reagent (Cat. No. B6916)
- BCA Reagent (Cat. No. BCA1)
- QuantiPro™ BCA Reagent (Cat. No. QPBCA)

### Storage/Stability

HIS-Select® Spin Columns are stable for at least two years at 2-8 °C.

### Precautions and Disclaimer

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

**Note:** Buffers or reagents that chelate metal ions should not be used with this product, since chelating agents may strip the metal ions off the silica. Strong reducing agents should also be avoided, since they may reduce the bound nickel and thus eliminate the binding of His-tagged proteins. See the Reagent Compatibility Table on page 3 for more information.

### Preparation Instructions

The HIS-Select® Spin Columns come ready-to-use. Each column is intended for one-time use and is not recommended for repeated applications.

Prepare the following buffers for purification of His-tagged recombinant proteins:

- Equilibration Buffer: 50 mM sodium phosphate, pH 8.0, with 0.3 M sodium chloride
- Wash Buffer: 50 mM sodium phosphate, pH 8.0, with 0.3 M sodium chloride and 5 mM imidazole
- Elution Buffer: 50 mM sodium phosphate, pH 8.0, with 0.3 M sodium chloride and 250 mM imidazole (available as Cat. No. H5413)

## Procedure

### I. Preparation of Protein Extract

The His-tagged recombinant protein may be purified from a crude cell extract or a partially purified protein fraction that has been 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.

CellLytic™ B or CellLytic™ Express is recommended for lysing *E. coli* cells. For optimal results, the pH of the protein sample buffer must be between pH 7.0-8.0.

### II. Purification

#### Native Conditions

The Equilibration and Wash Buffer should be supplemented with 1-10 mM imidazole and 0.15-0.5 M NaCl, to reduce non-specific protein binding. Due to the unique selectivity of the chelate, 5 mM imidazole in the Wash Buffer is sufficient to obtain high purity samples. Consult the Reagent Compatibility Chart for the use of other reagents.

1. Remove HIS-Select® Spin Column/collection tube from bag.
2. Add 600 µL of Equilibration Buffer to the spin column. Snap close collection tube lid onto spin column.
3. Centrifuge at 2,000-5,000 rpm (325-2,040 × *g*) in a microcentrifuge at room temperature for ~2 minutes.

**Note:** HIS-Select® Spin Columns may also be used with an appropriate vacuum manifold in place of the centrifugation step.

4. Remove spin column from collection tube.
5. Empty collection tube. Place spin column back in this same collection tube.
6. Load the prepared cell extract on the column. The column will hold up to 600 µL of extract at one time.
7. Centrifuge as in Step 3.
8. Remove spin column from collection tube. If needed, save the flow-through for later analysis.

9. Using a new collection tube, wash unbound protein from the spin column using 600 µL of Wash Buffer with centrifuging. Empty collection tube.
10. Repeat wash step with 600 µL of Wash Buffer one more time.
11. Using new collection tube, elute the target protein using up to 500 µL of Elution Buffer.
12. Centrifuge as in Step 3.
13. If necessary, analyze samples for protein using Bradford Reagent, BCA Reagent, QuantiPro™ BCA Reagent, SDS-PAGE, or Western blotting.

#### Denaturing Conditions

HIS-Select® Spin Columns can be used to purify proteins under denaturing conditions. If denaturing conditions are used, the protein will have to be solubilized in 8 M urea (such as Cat. No. U5378) or, if needed, CellLytic™ IB (Cat. No. C5236). The same purification procedure employed for native conditions can be used with denaturing buffers.

If using guanidine HCl, remove the guanidine prior to SDS-PAGE analysis by TCA precipitation, such as with Cat. No. 539180.

Examples of urea-containing, denaturing buffers follow:

- Equilibration Buffer: 0.1 M sodium phosphate, pH 8.0, with 8 M urea
- Wash Buffer: 0.1 M sodium phosphate, pH 8.0, with 8 M urea (same as Equilibration Buffer)
- Elution Buffer:
  - 0.1 M sodium phosphate, pH 4.5 to 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 made fresh daily.

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

## Reagent Compatibility Table

Reagent	Effect	Comments
Imidazole	Binds to the nickel-charged silica particles. Competes with the histidine-tagged proteins.	<ul style="list-style-type: none"> <li>No more than 10 mM is suggested in the extract, equilibration, and wash buffers to prevent non-specific binding of proteins.</li> <li>No more than 250 mM is suggested for the elution buffer.</li> <li>Many proteins will elute with imidazole levels in the range of 100-200 mM.</li> </ul>
Histidine	Binds to the nickel-charged silica particles. Competes with the histidine-tagged proteins.	<ul style="list-style-type: none"> <li>Can be used in place of imidazole in the extraction, equilibration, wash, and elution buffers.</li> <li>No more than 250 mM is suggested for the elution buffer.</li> </ul>
Chelating agents (EDTA and EGTA)	Strips nickel ions from the silica particles.	<b>Not recommended</b> as a buffer component, because of their ability to bind and remove nickel ions.
Guanidine HCl	Solubilizes proteins.	Use 6 M guanidine HCl to denature proteins.
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.	<ul style="list-style-type: none"> <li>50-100 mM buffer is recommended for purification with the spin columns.</li> <li>The pH range of the buffer should be between 7-8 with higher binding capacity occurring at the higher pH.</li> </ul>
Sodium Chloride	Prevents ionic interactions.	<ul style="list-style-type: none"> <li>Used in equilibration, wash, and elution buffers to help prevent binding of non-specific proteins to the spin column.</li> <li>Recommended levels are 0.3-0.5 M, but up to 2 M can be used.</li> </ul>
Glycerol	Helps stabilize proteins.	The equilibration, wash, and elution buffers may contain up to 50% glycerol.
2-Mercaptoethanol	Reducing agent used to reduce disulfide bonds.	<ul style="list-style-type: none"> <li>Add up to 20 mM in the extract buffer to reduce disulfide bonds.</li> <li>Higher levels may reduce the nickel ions on the silica particles.</li> </ul>
DTE and DTT	Reduces nickel ions.	<b>Not recommended.</b>
Nonionic detergents (TRITON™ and TWEEN®)	Help prevent non-specific binding.	Up to 2% may be used.
Glycine	Binds weakly to the filter plate. Competes weakly with histidine-containing proteins.	<b>Not recommended.</b> Use histidine or imidazole instead.

## References

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## Troubleshooting Guide

Problem	Possible Cause	Solution
His-tagged protein will not bind.	Incorrect binding conditions	<ul style="list-style-type: none"> <li>Verify pH and composition of sample and buffers.</li> <li>Make sure there are no chelating or reducing agents present.</li> </ul>
	His-tagged protein is not present.	Perform Western blot to determine if the His-tagged protein is present.
His-tagged protein elutes with wash buffer.	His-tag is buried within protein structure.	Perform purification under denaturing conditions.
	Wash conditions are too stringent.	<ul style="list-style-type: none"> <li>Decrease concentration of imidazole.</li> <li>Verify pH range of 7-8.</li> </ul>
His-tagged protein does not elute from the column.	His-tagged protein is buried within protein structure.	Perform purification under denaturing conditions.
	Elution conditions are too mild.	<ul style="list-style-type: none"> <li>Increase the amount of imidazole.</li> <li>For a denaturing purification, adjust elution buffer to pH 4.5 to make sure that the pH is low enough to elute the His-tagged protein.</li> </ul>
Non-specific proteins elute with the His-tagged protein.	Binding and wash conditions are too mild.	Increase the amount of imidazole in the extract and wash buffers up to 10 mM.
	Target protein is being degraded by proteases.	Add protease inhibitor cocktail <b>without chelating agents</b> (such as Cat. Nos. P8849, S8830, or PIC0004).
	Material is linked by disulfide bonds.	Add reducing agent, such as 2-mercaptoethanol up to 20 mM.
Protein precipitates during purification.	Temperature is too low.	Run columns at room temperature.
	Protein aggregates.	<ul style="list-style-type: none"> <li>Add stabilizing agents such as 5-10% glycerol, 0.1% TWEEN® 20 or TRITON™ X-100.</li> <li>Increase the sodium chloride concentration up to 2 M.</li> <li>Add reducing agent such as 2-mercaptoethanol up to 20 mM.</li> <li>Add metals or cofactors to stabilize the protein.</li> </ul>

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