Technical Bulletin

HIS-Select® Filter Plate

H0413

Product Description

The HIS-Select® Filter Plate is an immobilized metal-ion affinity chromatography (IMAC) product[1] that allows rapid purification of small-scale crude cell extracts that contain histidine-tagged (His-tagged) proteins. The HIS-Select® Filter Plate 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.

The HIS-Select® Filter Plate is selective for recombinant proteins with His-tags and exhibits very low non-specific binding of other proteins. The selectivity can be modulated with the inclusion of imidazole during chromatography. The binding capacity of the HIS-Select® Filter Plate is typically > 2 mg per well as determined with an ~30 kDa His-tagged protein.

Several publications[2-5] and dissertations[6] have cited use of this H0413 product in their research protocols.

Equipment Required but Not Provided

Suggested Cat. Nos. are provided as appropriate.

- Centrifuge or vacuum manifold
- Collection plates, such as Nunc™ 96 DeepWell™ Plates, 1 mL (Cat. No. P8241)
- Bacterial lysis buffer, such as CelLytic™ B (Cat. Nos. B7435, B7310, or C8740), CelLytic™ B Plus Kit (Cat. No. CB0500), or CelLytic™ Express (Cat. No. C1990)
- Imidazole, such as Cat. No. I5513
- Sodium chloride, such as Cat. No. S3014
- Sodium phosphate, such as Cat. No. S0751 (monobasic) and Cat. No. S3264 (dibasic)
- Bradford Reagent (Cat. No. B6916)
- BCA Reagent (Cat. No. BCA1)
- QuantiPro™ BCA Reagent (Cat. No. QPBCA)

Storage/Stability

The HIS-Select® Filter Plate is 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.

Preparation Instructions

The HIS-Select® Filter Plate comes ready-to-use. It 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 recombinant protein with a His-tag may be purified from a crude cell extract or a partially purified protein fraction prepared by standard techniques. The protein sample preparation steps should be empirically determined by the end user, since the conditions may vary depending on the nature of the recombinant protein and the host organism.

CelLytic™ B or CelLytic™ 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.

The Equilibration and Wash Buffers should be supplemented with 1-10 mM imidazole and 0.3-0.5 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.

Buffers or reagents that chelate metal ions should **not** be used with this product, since they may strip the metal ions from the particles. Strong reducing agents should also be avoided, since they may reduce the bound nickel ions and thus eliminate the binding of histidine-tagged proteins. Consult the Reagent Compatibility Chart for the use of other reagents.

II. Purification under Native Conditions

1. Place the HIS-Select® Filter Plate in a collection plate.
2. Add 600 µL of Equilibration Buffer to the wells of the filter plate.
3. Centrifuge at 2,000-5,000 rpm (1,100-6,850 × *g*) at room temperature for ~2 minutes.

   Note: The HIS-Select® Filter Plate may also be used with an appropriate vacuum manifold to replace the centrifugation step.
4. Empty the collection plate. Place the filter plate back in the same collection plate.
5. Load the prepared cell extract into the wells of the filter plate. Each well will hold up to 1.0 mL of extract at one time.
6. Centrifuge as in Step 3.
7. Remove the filter plate from the collection plate. If needed, save the flow-through for later analysis.
8. Using a new collection plate, wash unbound protein from the filter plate using 600 µL of Wash Buffer with centrifugation. Empty the collection plate.
9. Repeat the wash step (Step 8) with 600 µL of Wash Buffer.
10. Using a new collection plate, elute the target protein using up to 500 µL of Elution Buffer.
11. Centrifuge as in Step 3.
12. Elute the remaining target protein using another 500 µL of Elution Buffer. Centrifuge as in Step 3.
13. If necessary, analyze samples for protein using Bradford Reagent, BCA Reagent, QuantiPro™ BCA Reagent, or by SDS-PAGE or Western blotting.

III. Purification under Denaturing Conditions

The HIS-Select Filter Plate 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, CelLytic™ 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 tagged recombinant proteins will not elute in this range, try a pH as low as 4.5.

References

1. Koehn, J., and Hunt, I., "High-Throughput Protein Production (HTPP): A Review of Enabling Technologies to Expedite Protein Production", in *High Throughput Protein Expression and Purification* (S.A. Doyle, ed.). Humana Press (Totowa, NJ), p. 11 (2009).
2. Allen, B.D. *et al.*, *Proc. Natl. Acad. Sci. USA*, **107(46)**, 19838-19843 (2010).
3. Macdonald, I.K. *et al.*, *PLoS One*, **7(7)**, e40759 (2012).
4. Middleton, C.H. *et al.*, *PLoS One*, **9(8)**, e103867 (2014).
5. Wannier, T.M. *et al.*, *PLoS One*, **10(6)**, e0130582 (2015).
6. Nisthal, Alex, "Accelerating the Interplay Between Theory and Experiment in Protein Design". California Institute of Technology, Ph.D. dissertation, p. 197 (2012).

Reagent Compatibility Table

Reagent	Effect	Comments
Imidazole	Binds to the nickel-charged silica particles. Competes with the histidine-tagged proteins.	• 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 buffer. • Many proteins will elute with imidazole levels in the range of 100-200 mM.
Histidine	Binds to the nickel-charged silica particles. Competes with the histidine-tagged 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 buffer.
Chelating agents (EDTA and EGTA)	Strips nickel ions from the silica particles.	**Not recommended** 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.	• 50-100 mM buffer is recommended for purification with the filter plate. • The pH range of the buffer should be between 7-8 with higher binding capacity occurring 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 filter plate. • Recommended levels are 0.3-0.5 M, but up to 2 M can be used.
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.	• Add up to 20 mM in the extract buffer to reduce disulfide bonds. • Higher levels may reduce the nickel ions on the silica particles.
DTE and DTT	Reduces nickel ions.	**Not recommended.**
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.	**Not recommended.** Use histidine or imidazole instead.

Troubleshooting Guide

Problem	Possible Cause	Solution
His-tagged protein will not bind.	Incorrect binding conditions	• Verify pH and composition of sample and buffers. • Make sure there are no chelating or reducing agents present.
	His-tagged protein is not present.	Perform Western blot to determine if the His-tagged protein is present.
	His-tag is buried within protein structure.	Perform purification under denaturing conditions.
His-tagged protein elutes with wash buffer.	Wash conditions are too stringent.	• Decrease concentration of imidazole. • Verify pH range of 7-8.
	His-tagged protein is buried within protein structure.	Perform purification under denaturing conditions.
His-tagged protein does not elute from the column.	Elution conditions are too mild.	• Increase the amount of imidazole. • 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.
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.
Protein precipitates during purification.	Temperature is too low.	Run columns at room temperature.
	Protein aggregates.	• Add stabilizing agents such as 5-10% glycerol, 0.1% TWEEN® 20 or TRITON™ X-100. • 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 the protein.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.