3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

HIS-Select® High Capacity (HC) Nickel Coated Plate, 96-well, clear plate

Catalog Number **S5563**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Immobilized metal affinity chromatography (IMAC) is widely used for the purification and identification of recombinant fusion proteins with histidine tags. The affinity of the histidine tag for the nickel chelate is sequence dependent but is generally very high. This allows the histidine-containing protein to be captured on a solid support (agarose, multiwell plate, magnetic beads, etc.) that contains a chelated nickel ion.[1-3]

HIS-Select® HC 96-well plates are coated with a proprietary high-density nickel chelate matrix. This matrix provides high capacity affinity binding of recombinant fusion proteins with histidine tags. The high capacity coating allows unique multi-sample application potential including:

- Affinity purification of recombinant fusion proteins with histidine tags
- Direct quantitation of bound protein by standard protein methods e.g., BCA or Bradford (Coomassie® blue)
- Direct transfer of protein for PAGE and Western blotting, or for mass spectrometry

The unique affinity matrix coating possesses the following qualities:

- Highly specific interaction providing single step protein purification to greater than 90% purity
- High capacity of ≥4 μg protein per well
- Recharged with nickel as a highly stable chelate
- Robust coating allows multiple usage and may be regenerated
- High resistance to commonly used detergents (see Table 1)

Reaction Volume

The HIS-Select binding surface is coated at a reaction volume of 200 μl/well. The HIS-Select coating minimizes non-specific binding.

Binding Capacity

In saturation binding assays performed using this product, binding ≥4 μg protein per well is observed following 1 hour incubation at 37 °C using a control protein containing histidine.

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.

Storage/Stability

For optimal performance, the unopened product should be stored in a dry place at 2–8 °C. Under these storage conditions, the product is stable for two years. For short-term storage of less than 3 months, the product may be stored at room temperature. Once opened, it is suggested that the product be used within one week.

Not recommended for assays at >60 °C.

Procedure

Binding of the histidine-containing proteins is highly pH dependent. The suggested pH range for incubation is between 7.0–7.5, however binding can occur over the range of pH 6.5–8.0. Binding to the plates may be accomplished in the presence of most detergents, chaotropic agents and reducing agents (see Table 1).

Binding of Recombinant Fusion Proteins Containing Histidine: purification from a crude cell extract

1. Prepare the cell extract by standard protocols depending on cell type (see Table 2 for compatibility). Good extractions have been obtained using CelLytic™ B (Catalog Number B7435) in 0.15 M NaCl, 0.01 M Tris-HCl pH 7.5 (TBS). Binding is concentration-dependent and good results will be obtained if the target fusion protein is at least 0.1 mg/ml in the clarified extract.

2. Load up to 0.2 ml extract per well. Allow the samples to incubate 1–2 hours at 37 °C, or up to 4 hours at 18–30 °C, or, as convenient, overnight (12 hours) at 2–8 °C. Binding is time-dependent and saturation may not be attained at the shorter incubation times.
   Note: It is recommended to cover plate during incubations to minimize evaporation and contamination.

3. Wash the wells a minimum of three times with 0.25–0.3 ml per well of TBS containing 0.05% TWEEN® 20 (Catalog Number T9039). Phosphate buffered saline containing 0.05% TWEEN 20 (Catalog Number P3563) may also be used with good results.
   Notes: If desired, a direct protein quantitation may be performed on bound protein in the wells using bicinchoninic acid (BCA) solution (Catalog Number B9643) or Bradford reagent (Catalog Number B6916) with protein standards at 1–20 μg per well.

   A second incubation may be performed with a solution containing a compound or protein that is complementary to the captured protein to form a complex. Parameters may be followed as outlined in steps 2 and 3.

4. Elution of the bound protein(s) may be efficiently accomplished by incubation with 0.2 ml per well of TBS or PBS containing 0.1 M imidazole pH 7.5 for 1–5 minutes. Depending on analyses being used to characterize the proteins, other elution conditions may be more appropriately utilized (see Table 2). As an example, for subsequent analysis by mass spectrometry, good results have been attained using 0.1 % triflouroacetic acid (TFA).
   Note: Depending on the elution conditions, the plates may be used several times without significantly diminishing subsequent performance.

5. Characterization of the eluted protein (or protein complex) may be accomplished by a variety of methods requiring 2–10 μg protein. As examples, samples obtained in the eluant have been loaded directly onto SDS-PAGE mini-gels or diluted in matrix components for mass spectrometry. To increase loading density for PAGE, TCA precipitation of the well eluant has been performed.

Table 1.
Compatibility Table - Listed Reagents are compatible at the indicated concentrations

Reagent	Concentration	Comment
TRITON® X-100, SDS, TWEEN 20	0.1–1%	
Guanidine-HCl	3 M	There is competitive inhibition at higher concentrations
Urea	4 M	There is competitive inhibition at higher concentrations
Imidazole	<1 mM	A strongly competitive inhibitor; may be used to minimize binding of proteins containing histidine
2-Mercaptoethanol (2 ME)	5–10 mM	Higher concentrations may eventually reduce nickel
Dithiothreitol (DTT)	0.5–1.0 mM	Higher concentrations may eventually reduce nickel. 2-ME recommended for longer incubations.

Table 2.
Elution Table -Listed Reagents provide greater than 95% recovery at the indicated concentrations

Reagents	Concentration	Comment
Imidazole	>50 mM	A strongly competitive inhibitor. Conveniently used at 100 mM in TBS or PBS, pH 7.5.
EDTA	100 mM	A competitive chelator that will strip nickel from plate matrix.
TFA	0.1%	Harsh elution conditions. Compatible with matrix reagent for mass spectrometry.
Acetic Acid	2 M	

Optimization of Results

Optimal results may be obtained when the protein for capture possesses an accessible fusion tag containing histidine. To increase accessibility, it may be necessary to include denaturing agents in the incubation buffer (see Table 1). Proteins containing histidine may compete for binding and 1.0 mM imidiazole may be included in the incubation buffer to minimize interference. Longer incubation times may be necessary for efficient capture at low protein concentrations.

In addition, other standard measures may be evaluated to decrease nonspecific binding. Users are encouraged to modify buffers with components in the concentration ranges stated in Table 3.

Table 3.
Buffer Components

Component	Concentration
Detergents	0.05–0.5% TWEEN 20 or CHAPS
Salt	0.5–1.0 M NaCl
Protein blockers	0.1–0.5% Gelatin 0.1% BSA or casein
Non-protein blockers	1% Polyvinyl alcohol (PVA) or Polyvinylpyrrolidone

References

1. Sulkowski, E, Immobilized Metal Ion Affinity Chromatography of Proteins. In Protein Purification: Micro to Macro, R. Burgess, (Ed.), pp. 149-162 (Alan R Liss, Inc, New York, 1987).
2. Hemdan, ES: et al., Surface Topography of Histidine Residues: A Facile Probe by Immobilized Metal Ion Affinity Chromatography. Proc. Natl. Acad. Sci. USA, **86**, 1811-1815 (1989).
3. Andersson, L., et al., Facile Resolution of α-Fetoproteins and Serum Albumins by Immobilized Metal Affinity Chromatography. Cancer Res., **47**, 3624-3626 (1987).

CS,JD,KMR,MAM 08/10-1