

## Product Information

### **HIS-Select HS Nickel-Coated 96 Well Plate (Clear)** (High Sensitivity Plate)

Product Codes **S 5688 (Strip Plate)**  
**S 9191 (Solid Clear Plate)**

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-containing 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.<sup>1-3</sup> HIS-Select HS Nickel coated plates are designed for low level detection of recombinant fusion proteins with histidine-containing tags. The multiwell plate format allows multiple samples to be screened simultaneously. The captured proteins can be detected using standard enzyme-linked assay (ELA) techniques.<sup>4</sup>

Binding of histidine-containing fusion proteins is highly pH dependent. The suggested pH range for incubation is between 7.0 to 7.5; however, binding can occur over the range of pH 6.5 to 8.0. Binding to the plates may be accomplished in the presence of most detergents, chaotropic agents, and reducing agents. Imidazole may be added at  $\leq 5$  mM during incubation to reduce non-specific binding of protein. Strongly anionic detergents (e.g., SDS) are not recommended for this plate format. A chelating agent, such as EDTA at  $\geq 15$  mM, or a histidine mimic, such as imidazole at  $\geq 250$  mM, may be added to demonstrate specificity since these agents block the binding of the histidine-containing compound.

### Reaction Volume

The HIS-Select HS Nickel binding surface is coated at a reaction volume of 200  $\mu$ l/well. This coating minimizes non-specific binding.

### Sensitivity

In binding assays performed using this product,  $\leq 1$  ng per well of a recombinant fusion protein with a histidine-containing tag can be detected.

### **Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses.

### **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.

The plates are not recommended for assays at  $> 60$  °C.

### **Procedure**

Binding of Recombinant Fusion Proteins with Histidine-Containing Tags: cell lysate screening or capture of purified protein

1. Prepare a series of dilutions of the histidine-containing fusion protein or peptide in Tris or phosphate buffered saline (TBS, Product Code T 6664, or PBS, Product Code P 3813) pH 7.0 to 7.5. It may be beneficial to include a blocking reagent to minimize nonspecific binding especially when using a cell lysate; see the Optimization of ELISA Results section for guidance. Starting protein concentrations between 0.5 to 5  $\mu$ g/ml should be used if the optimal concentration is not known. For cell extracts with expression levels at approximately 10 mg/L culture or 0.5 mg per g of cell paste, use 5 ml of extraction buffer per g of cell paste and then dilute the extract 1:100 to 1:1000. HIS-Select HS plates are compatible with most cell lysis reagents including CellLytic B (Product Code B 3553) and commonly used detergents.

2. Add up to 200  $\mu$ l per well of each dilution. Use at least 3 wells per sample. As a blank to detect the background signal, add the same volume of diluent alone to at least 3 wells. Allow the samples to incubate for 30 minutes to 4 hours at a temperature between 18–30 °C. Longer incubations may be necessary for low concentrations of histidine-containing fusion proteins.
3. Wash the wells three times, 300  $\mu$ l per well, with TBS or PBS containing 0.05 % TWEEN® 20 (TBST, Product Code T 9039, or PBST, Product Code P 3563).
4. Incubate the samples with up to 200  $\mu$ l per well of an appropriately diluted primary antibody in PBS or TBS containing 0.05 % TWEEN 20 for 30 minutes to 1 hour. A blocking agent may be included in the diluent to optimize signal to background ratio.
5. Wash the wells three times, 300  $\mu$ l per well, with PBS or TBS containing 0.05 % TWEEN 20.
6. Incubate the samples with up to 200  $\mu$ l per well of an appropriately diluted enzyme-labeled secondary antibody in PBS or TBS containing 0.05 % TWEEN 20 for 30 minutes to 1 hour. A blocking agent may be included in the diluent to optimize signal to background ratio.
7. Wash the wells three times, 300  $\mu$ l per well, with PBS or TBS containing 0.05 % TWEEN 20.
8. Detect the bound protein with a colorimetric substrate appropriate for the detection enzyme used.

#### **Troubleshooting: Optimization of ELISA Results**

There are four major areas where detection of the target protein can be optimized: nonspecific binding, wash conditions, antibody affinity, and conjugate concentration.

#### Nonspecific Binding:

Factors that contribute to nonspecific binding are ionic interactions, hydrophobic interactions, and cross-reactivity. To reduce nonspecific binding, changes in conjugate concentrations and wash buffers can be made. Users are encouraged to modify buffers with components in the concentration ranges indicated in Table 1.

**Table 1.**  
Buffer Components

Detergents	0.05–0.5% TWEEN 20 0.05–0.5% CHAPS
Salt	0.5–1.0 M NaCl
Protein blockers (Higher levels of BSA are not recommended)	0.05% BSA or casein 0.1–0.5% Gelatin
Non-protein blockers	1% Polyvinyl alcohol or Polyvinylpyrrolidone

#### Wash Conditions:

To limit reversible nonspecific binding interactions, at least three wash steps are recommended.

#### Antibodies and Conjugates:

For optimal signal performance, the user is encouraged to use high affinity antibodies and conjugates. Commercially obtained antibodies and conjugates should be used at the concentrations suggested by the supplier.

Also see the Troubleshooting Guide on following page.

#### **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  $\alpha$ -Fetoproteins and Serum Albumins by Immobilized Metal Affinity Chromatography. Cancer Res., **47**, 3624-3626 (1987).
4. Crowther, JR: ed, "ELISA: Theory and Practice", Methods in Molecular Biology, **Vol. 42** (Humana Press, Totowa, NJ, 1995) Product No. Z36,415-0

## Troubleshooting Guide

Problem	Cause	Solution
High background	Nonspecific binding of protein in target protein solution	Decrease the concentration of target protein solution. Add blocking agents, salt, and/or detergents to diluent. Add 1 to 4 mM imidazole in diluent
	Nonspecific binding of detection conjugates	Add blocking agent, salt, or detergents to conjugate diluents. Use higher affinity antibodies.
	Insufficient washing between incubation steps	Wash wells with 300 µl per well, 3 to 6 times, using buffer containing at least 0.05 % detergent. Increase soaking time with wash solution.
	Detection conjugates too concentrated	Dilute conjugates
	Substrate contamination/ degradation	Use freshly-prepared substrate solutions. Avoid repeated temperature fluctuations of pre-made substrates.
Low signal	Insufficient target protein captured.	Increase the concentration of target protein solution. Increase incubation time with target protein. Add detergent to buffer containing the target protein to increase accessibility of the histidine-containing tag. Ensure that pH of the target protein is between 6.5 to 8 and does not contain EDTA or > 5 mM imidazole.
	Detection conjugate concentration is too low.	Increase the concentration of conjugate solutions.
	BSA concentration is too high in the diluent.	Use diluents containing 0.05 % BSA or less. Use gelatin as a substitute for BSA.
	Substrate kinetics slow	Increase incubation time with substrate. Use substrate that has been warmed to room temperature. Switch to more sensitive substrate (e.g., when using peroxidase for detection, use TMB instead of ABTS)

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