INSTRUCTIONS

HisGrab™ Nickel Coated 96-Well Plates

PIERCE
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

15142 15242 15342 15442

0690.3

Number	Description
15142	**HisGrab™ Nickel Coated Plates (clear, 8-well strip),** 5 plates/package
15242	**HisGrab™ Nickel Coated Plates (white, 96-well)** 5 plates/package
15342	**HisGrab™ Nickel Coated Plates (black, 96-well),** 5 plates/package
15442	**HisGrab™ Nickel Coated Plates (clear, 96-well),** 5 plates/package

Activation level: 200 µl

Binding Capacity: ~9 pmol His-tagged protein (27 Da)/well

Note: Plates are supplied pre-blocked with bovine serum albumin.

Storage: Upon receipt store plates at 4°C in unopened pouches. Once opened, place unused plates in a resealable bag with desiccant and store at 4°C.

Introduction

HisGrab™ Nickel Coated Plates are ideal for analyzing polyhistidine-tagged fusion proteins by ELISA-based methods. Proteins that contain a succession of several histidine residues at the amino or carboxyl terminus have a strong binding affinity for metal. Bacterial lysates containing polyhistidine-tagged fusion proteins can be added directly to the plates without the need for blocking. The clear, white or black plates can be used with colorimetric, chemiluminescent or fluorescent detection methods, respectively.

Important Product Information

- His-tagged protein binding to metal may be related to topology, microenvironment, reversible reactions, pH, ionic strength, and other factors. Protein folding may hinder binding of the histidine tag. Binding can occur in chaotropic agents such as 8 M urea, 6 M guanidine•HCl, or 3 M thiocyanate. Nickel binding requires the presence of at least two histidine residues.
- A variety of solutes are compatible with metal interactions with macromolecules, including nonionic detergents, ethylene glycol and dimethylsulfoxide. Sodium chloride may enhance or decrease affinity.
- Avoid using solutions that contain metal chelators such as EDTA. Also avoid reducing agents such as mercaptoethanol. High concentrations of imidazole are commonly used to elute nickel-bound his-tagged proteins and, therefore, should be avoided for binding reactions.

General ELISA Procedure

A. Materials

- Cell lysate containing polyhistidine-tagged fusion protein
- Dilution Buffer for lysate: Tris-buffered saline (TBS, Product No. 28376) or phosphate-buffered saline (PBS, Product No. 28374)
- Wash Buffer: Dilution Buffer containing 0.05% Tween®-20 (Product No. 28320)
- Antibody to poly-histidine-tagged fusion protein
- Enzyme-conjugated or fluorescent-labeled secondary antibody
- Enzyme Substrate (e.g., for HRP use TMB Substrate Kit, Product No. 34021)

B. Method

Note: Plates are supplied pre-blocked with bovine serum albumin.

1. Dilute lysate with Dilution Buffer. Add 100 µl of diluted lysate per well and incubate with shaking for 1 hour at room temperature.
2. Wash wells three times using 200 µl of Wash Buffer for each wash.
3. Add 100 µl of primary antibody per well and incubate for 1 hour at room temperature.
4. Wash wells three times using 200 µl of Wash Buffer for each wash.
5. Add 100 µl per well of secondary antibody. Incubate for 1 hour at room temperature.
6. Wash wells three times using 200 µl of Wash Buffer for each wash.
7. Develop and evaluate plate.

Related Pierce Products

37070	**SuperSignal® ELISA Pico Chemiluminescent Substrate,** 100 ml, peroxidase substrate
15169	**QuantaBlu™ Fluorogenic Peroxidase Substrate Kit**
34028	**1-Step™ Ultra TMB-ELISA,** 250 ml, colorimetric peroxidase substrate
37621	**1-Step™ PNPP,** 100 ml, colorimetric phosphatase substrate
15075	**ImmunoWare™ Reagent Reservoirs,** 200/pkg.
15082	**ImmunoWare™ Microtube Racked System,** 960 tubes
15036	**Sealing Tape for 96-Well Plates,** 100/pkg.
78248	**B-PER® Bacterial Protein Extraction Reagent,** 500 ml
78410	**Halt™ Protease Inhibitor Cocktail, EDTA-Free**, 1 ml, sufficient for 100 ml of extract
78101	**Immobilized Nickel Chelated Column,** 1 ml
15165	**HisProbe™-HRP,** 2 mg
15168	**SuperSignal® HisProbe™ Western Blotting Kit**
78100	**B-PER® 6xHis Fusion Protein Purification Kit**

References

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Riggs, P., in Ausubel. F.M. *et al*. (eds), (1990). *Current Protocols in Molecular Biology*. 16.4.1-16.6.14.

Janknecht, Ralf *et al*. (1991). Rapid and efficient purification of native histidine-tagged protein expressed by recombinant vaccinia virus. *Proc. Natl. Acad. Sci.USA* **88:**8972-6.

Jin, L., *et al.* (1995). Use of a *N,N*-bis[carboxymethyl]lysine-modified peroxidase in immunoassays. *Anal. Biochem*. **229:**54-60.

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Belew, M., and Porath, J. (1990). Immobilized metal ion affinity chromatography. Effect of solute structure, ligand density, and salt concentration on the retention of peptides. *J. Chrom*. **516:**333-354.

Hemdan, E., *et al.* (1989). Surface topography of histidine residues: a facile probe by immobilized metal ion affinity chromatography. *Proc. Natl. Acad. Sci*. USA **86:**1811-5.

Current versions of product instructions are available at www.piercenet.com. For a faxed copy, call 800-874-3723 or contact your local distributor.