INSTRUCTIONS HisGrabTM Nickel Coated 96-Well Plates



15142 15242 15342 15442

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

HisGrabTM 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)

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Warranty: Pierce Biotechnology products are warranted to meet stated product specifications and to conform to label descriptions when stored and used properly. Unless otherwise stated, this warranty is limited to one year from date of sale when used according to product instructions. Pierce Biotechnology's sole liability for the product is limited to replacement of the product or refund of the purchase price. Unless otherwise expressly authorized in writing by Pierce Biotechnology, Pierce products are supplied for Research Use Only and are intended to be used by a technically qualified individual. Pierce Biotechnology's quality system is certified to ISO 9001. Pierce Biotechnology products are not produced in accordance with FDA's current Good Manufacturing Practices. Pierce Biotechnology strives for 100% customer satisfaction. If you are not satisfied with the performance of a Pierce Biotechnology product, please contact Pierce Biotechnology or your local distributor.



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 TM 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 TM Protease Inhibitor Cocktail, EDTA-Free, 1 ml, sufficient for 100 ml of extract
78101	Immobilized Nickel Chelated Column, 1 ml
15165	HisProbe TM -HRP, 2 mg
15168	SuperSignal [®] HisProbe [™] Western Blotting Kit
78100	B-PER [®] 6xHis Fusion Protein Purification Kit

References

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SuperSignal[®] Technology is protected by U.S. Patent # 6,432,662.

QuantaBlu[™] Technology is protected by U.S. Patent # 6,040,150 and # 6,437,179.

B-PER® Technology is protected by U.S. Patent # 6,174,704.

Current versions of product instructions are available at <u>www.piercenet.com</u>. For a faxed copy, call 800-874-3723 or contact your local distributor. ©Pierce Biotechnology, Inc. 3/2006. Printed in the USA.