

Penta·His™ Mouse Monoclonal Antibody (Anti-Pentahistidine) (P-21315)

Quick Facts

Storage upon receipt:

- 4°C
- Avoid freeze–thaw cycles

Introduction

The oligohistidine domain is a Ni²⁺-binding peptide sequence comprising a string of four to six histidine residues. When the DNA sequence corresponding to the oligohistidine domain is fused in frame with a gene of interest, the resulting fusion protein can be easily purified using a nickel-chelating resin. The Penta·His™ monoclonal IgG₁ antibody provides a sensitive method for specific detection of fusion proteins that have an oligohistidine domain comprising five or six consecutive histidine residues. The antibody does not recognize tetrahistidine domains or domains in which the histidine string is interrupted by another amino acid. The antibody binds to the oligohistidine domain regardless of the surrounding amino acid context and even when the group is partially hidden, although subtle differences in the amino acid context may change the sensitivity limit for a particular fusion protein. This antibody can be used to detect oligohistidine fusion proteins on Western blots, dot blots or colony blots, or in tissues.

Materials

Contents

Penta·His mouse monoclonal antibody is provided in a 100 µg unit size. The antibody was lyophilized from a solution containing phosphate-buffered saline (PBS), sucrose, poly(ethylene glycol) and 0.08% sodium azide. Sufficient material is supplied to stain 50–100 minigel blots (8 cm × 10 cm).

Storage and Handling

Upon receipt, the lyophilized antibody should be stored at 4°C. When properly stored, this product is stable for at least one year.

To prepare a stock solution, reconstitute the antibody in 0.5 mL of deionized water (dH₂O) to a final concentration of 0.2 mg/mL. Store the solution for up to 3 months at 4°C. For storage up to 6 months, divide the solution into single-use aliquots and freeze at -20°C. AVOID REPEATED FREEZING AND THAWING.

Applications

General information on the use of this product for blotting or immunohistochemical applications is provided below. This product is offered in collaboration with QIAGEN. For more detailed information on the use of this product, technical information is available online at www.qiagen.com.

Blotting Applications

Western and dot blot preparation. For Western blots, prepare samples for SDS-polyacrylamide gel electrophoresis using standard procedures. After separation of the proteins by electrophoresis, transfer the proteins onto a PVDF membrane using standard procedures, and dry the blot. For dot blots, simply spot the protein solution directly onto a PVDF membrane.

Colony blot preparation. Colony blots can be used to identify bacterial colonies that express an oligohistidine fusion protein. Grow the colonies on the appropriate medium overnight using standard procedures. Place a nitrocellulose filter on top of the colonies, mark the filter and pull the filter off. Place the filter, colony side up, onto fresh agar containing the appropriate antibiotics and inducers. After induction, lyse the colonies on the nitrocellulose filter by placing the filter, colony side up, onto a stack of cellulose filter papers soaked with the appropriate solutions, using standard procedures.

Immunodetection for blots. For immunodetection of oligohistidine fusion proteins on blotting membranes, use the Penta·His antibody at a final concentration of between 0.1 µg/mL and 0.2 µg/mL (a 1:1000–1:2000 dilution of the stock solution). Standard buffers and blocking agents, such as 3% BSA (bovine serum albumin) may be used; however, milk powder should not be used, as it reduces sensitivity. Denaturation of the proteins is recommended to ensure exposure of the oligohistidine domain. Detect the Penta·His antibody with an anti–mouse IgG secondary antibody conjugated to either alkaline phosphatase or horseradish peroxidase. The enzyme activity can then be visualized using a chromogenic, fluorogenic or chemiluminescent substrate. Molecular Probes provides several kits that are convenient for detection of the Penta·His antibody on Western blots. Pro-Q™ Western Blot Stain Kits #1 and #2 and DyeChrome™ Western Blot Stain Kits #1 and #4 contain an anti–mouse IgG–alkaline phosphatase conjugate and a fluorogenic substrate for detection of the alkaline phosphatase. Chemiluminescent substrates are not recommended for colony blots.

Cell and Tissue Staining

Immunohistochemical methods can be used to investigate the location of recombinant proteins expressed in particular cells within tissues or organs, as well as for determination of the sub-cellular localization of proteins after targeting to particular cell compartments. The Penta-His antibody has been used to detect a hexahistidine aggrecan fusion protein in the perinuclear Golgi and the endoplasmic reticulum of transiently transfected CHO cells.¹ The technique can also be used to localize cellular proteins that interact with hexahistidine fusion proteins.²

Preparation of the cells or tissue. Fix the tissue with 2% para-formaldehyde and permeabilize with 0.25% Triton[®] X-100. This relatively mild fixation protocol best preserves epitopes and

best maintains the position of the protein within the cellular structure.

Immunodetection for cells and tissues. Before applying the antibody to the tissue, block nonspecific binding sites using 5% bovine serum albumin (BSA). For immunodetection of the oligohistidine fusion protein, use the Penta-His antibody at a final concentration of 4–10 µg/mL (a 1:20–1:50 dilution of the stock solution). Detect the Penta-His antibody with an anti-mouse IgG secondary antibody conjugated to a fluorescent dye or to alkaline phosphatase or horseradish peroxidase. The fluorescent dye can be seen directly using a fluorescence-based microscope. The enzyme activity can be detected using a chromogenic or fluorogenic substrate.

References

1. QIAGEN News 1999 No. 1, 3 (1999); 2. Neuroendocrinology 65, 223 (1997).

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — www.probes.com — for the most up-to-date information

Molecular Probes, Inc.

PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 • Fax: (541) 344-6504

Customer Service: 7:00 am to 5:00 pm (Pacific Time)

Phone: (541) 465-8338 • Fax: (541) 344-6504 • order@probes.com

Toll-Free Ordering for USA and Canada:

Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)

Phone: (541) 465-8353 • Fax: (541) 465-4593 • tech@probes.com

Molecular Probes Europe BV

PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)

Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)

Phone: +31-71-5233431 • Fax: +31-71-5241883
eurotech@probes.nl

Molecular Probes' products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Penta-His antibody is provided under license from QIAGEN for research use only and is covered by patent DE 195 07 166; international patent applications are filed under W09626963. Penta-His is a trademark of QIAGEN.

Several of Molecular Probes' products and product applications are covered by U.S. and foreign patents and patents pending. Our products are not available for resale or other commercial uses without a specific agreement from Molecular Probes, Inc. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to busdev@probes.com. All names containing the designation [®] are registered with the U.S. Patent and Trademark Office.

Copyright 2001, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.