GoldiBlot™ His Western Blot Kit

Nanoprobes

95 Horseblock Road, Unit 1, Yaphank NY 11980-9710
Tel: (877) 447-6266 (Toll-Free in US) or (631) 205-9490 Fax: (631) 205-9493
Tech Support: (631) 205-9492 tech@nanoprobes.com
www.nanoprobes.com

PRODUCT INFORMATION

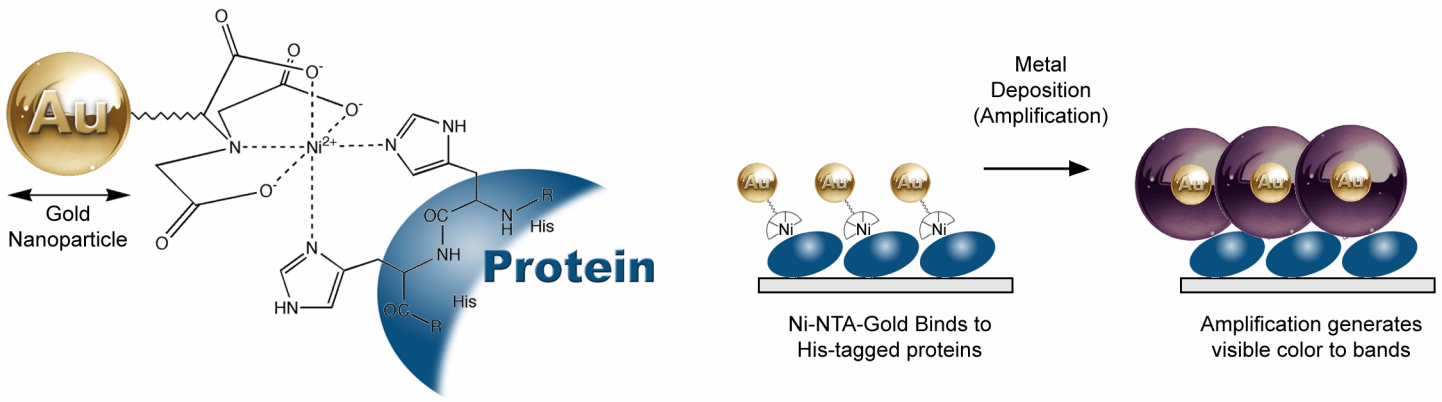
Product Name: GoldiBlot™ His Western Blot Kit
Catalog Number: 2090 and 2090A
Revision: 1.0 (February 2008)

INTENDED USE

GoldiBlot™ His Western Blot Kit is intended and optimized for direct visualization of recombinant His-tagged proteins and other proteins bearing different histidine tags in western or dot blotting applications. Unlike detection by anti-6xHis antibodies, GoldiBlot™ His detection does not require a specific location of the polyhistidine tag (*N*- or *C*-terminus), does not need primary and secondary antibody incubations, or the presence of specific adjacent amino acid sequences.

PRINCIPLE OF GoldiBlot™ HIS WESTERN BLOT KIT

GoldiBlot™ His Western Blot Kit uses Ni-NTA (nickel-nitrilotriacetic acid)-functionalized gold nanoparticles to specifically bind to His-tagged proteins.[1-6] With the autometallographic amplification subsequently applied to the gold nanoparticles, GoldiBlot™ allows the direct visualization of His-tagged proteins. GoldiBlot™ generates specific purple colored metallic bands or dots which do not fade and will not dissolve in water and organic solvents. The GoldiBlot™ His Western Blot Kit can detect nanogram levels of purified His-tagged proteins. The entire procedure takes about 1 hour.



(left) Ni-NTA-Gold, showing mechanism of binding to a polyhistidine (His) – tagged protein. (right) Principle of GoldiBlot™: gold binding followed by autometalographic amplification (deposition of metal selectively onto the gold particles) generates visible signal.

REAGENTS PROVIDED

The following materials are sufficient for 15 mini-blots or 900 cm^2 of membrane

GoldiBlot™ Nickel-NTA-Gold	1.5 mL
GoldiBlot™ AutoMet Detect A	40 mL
GoldiBlot™ AutoMet Detect B	40 mL
GoldiBlot™ AutoMet Detect C	40 mL
GoldiBlot™ AutoMet Detect D	40 mL

MATERIALS REQUIRED, BUT NOT SUPPLIED

1. Tris Buffered Saline Tween® -20 (**TBST**): 20 mM Tris, 0.15 M NaCl, pH7.6, 0.1% (w/v) Tween® -20
2. 5 % (w/v) nonfat dried milk in TBST
3. Tris Buffered High Salt Saline Tween® -20 (**TBST-High NaCl**): 20 mM Tris, 1.5 M NaCl, pH7.6, 1% (w/v) Tween® -20
4. 1 % (w/v) nonfat dried milk in TBST-High NaCl
5. 10 mM imidazole in TBST-High NaCl

STORAGE

Refrigerate at 4°C. The product is shipped at ambient temperature.

PROCEDURE FOR DETECTION OF POLYHISTIDINE-TAGGED PROTEINS

Note: Volumes indicated below are for one 7 x 8.4 cm^2 blot. Volumes can be adjusted for staining multiple blots or for different sized blots.

All GoldiBlot™ reagents and other required materials should be equilibrated to room temperature prior to the blotting procedure. All incubations of the Goldiblot™ western blotting should be performed at room temperature with gentle shaking, preferably using an orbtal shaker to ensure that the membrane remains immersed.

1. Transfer protein from gel to a PVDF membrane.

   Note: Although other membranes can be used, optimization may be required.

2. Place the membrane in a tray and equilibrate with TBST for 2 minutes.

3. Block the membrane with 5 % (w/v) nonfat dry milk in TBST for 12 minutes.

4. Place the membrane in 10 mL of 1 % (w/v) nonfat dried milk in TBST-High NaCl. Add 0.1 mL of GoldiBlot™ Nickel-NTA-Au to the 10 mL of Milk/TBST-High NaCl. Incubate the blot for 20 minutes.

5. Wash the membrane three times with 15 mL of 10 mM imidazole in TBST-High NaCl for 3 minutes each.

6. Wash the membrane three times with 15 mL of deionized water for 3 minutes each.

7. Before starting the last deionized water wash, mix 2.5 mL GoldiBlot™ AutoMet Detect A with 2.5 mL B in a clean 15 mL container. After 5 minutes, add 2.5 mL C and 2.5 mL D to the mixture of A and B, and mix. Incubate the blot with 10 mL of the ABCD mixture for 6 to 15 minutes, or until satisfactory staining is reached.

   Note: The incubation time of GoldiBlot™ AutoMet Detect ABCD depends on the quantities of His-tagged proteins loaded. The bands loaded with more than 100 ng His-tagged proteins can be seen within 6 minutes. Longer incubation time may be needed in order to see less than 20 ng His-tagged proteins. However, longer incubation may lead to the visualization of some non specific background bindings.

8. Wash the membrane three times with 15 mL of deionized water for 3 minutes each to terminate the autometallographic amplification.

   Note: any light purple color membrane background will fade away as the membrane dries out.

9. Air-dry the membrane.

Note: The concentration of NaCl and Tween 20 in TBST-High NaCl (used in GoldiBlot™ Nickel-NTA-Au binding and imidazole washes) can be slightly adjusted to achieve an optimized signal-to-noise ratio. Less NaCl and Tween-20 can enhance the band intensity of His-tagged proteins, and higher NaCl and Tween 20 help reduce the non specific background staining.

REFERENCES

1. Hochuli, E.; Dobeli H, Schacher A. New metal chelate adsorbent selective for proteins and peptides containing neighbouring histidine residues. *J. Chromatograph.*, **411**, 177-184 (1987).
2. Schmitt, J.; Hess, H., and Stunnenberg, H. G.: Affinity purification of histidine-tagged proteins. *Molecular Biology Reports*, **18**, 223-230 (1993).
3. Hainfeld, J. F.; Liu, W.; Halsey, C. M. R.; Freimuth, P., and Powell, R. D.: Ni-NTA-Gold clusters target His-tagged proteins. *J. Struct. Biol.*, **127**, 185-198 (1999).
4. Collins, R. F.; Beis, K.; Clarke, B. R.; Ford, R. C.; Hulley, M.; Naismith, J. H.; and Whitfield, C.: Periplasmic protein-protein contacts in the inner membrane protein Wzc form a tetrameric complex required for the assembly of Escherichia coli group 1 capsules. *J. Biol. Chem.*, **281**, 2144-2150 (2006).
5. Wolfe, C. L.; Warrington, J. A.; Treadwell, L., and Norcum, M. T.: A three-dimensional working model of the multienzyme complex of aminoacyl-tRNA synthetases based on electron microscopic placements of tRNA and proteins. *J. Biol. Chem.*, **280**, 38870-38878 (2005).
6. Bumba, L.; Tichy, M.; Dobakova, M.; Komenda, J., and Vacha, F.: Localization of the PsbH subunit in photosystem II from the Synechocystis 6803 using the His-tagged NiNTA Nanogold labeling. *J. Struct. Biol.*, **152**, 28-35 (2005).