95 Horseblock Road, Unit 1
Yaphank, NY 11980-9710 USA
Tel: 631-205-9490 Fax: 631-205-9493
Tech Support: tech@nanoprobes.com 631-205-9492
nano@nanoprobes.com www.nanoprobes.com

GoldiBlot™ His-Tag Western Blot Kit

PRODUCT INFORMATION

Product Name: GoldiBlot™ His-Tag Western Blot Kit
Catalog Number: 2090 and 2090A
Revision: 1.2 (June 2014)

INTENDED USE

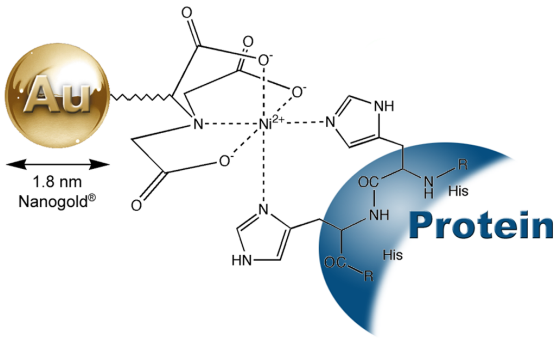
GoldiBlot™ His-Tag Western Blot Kit is intended and optimized for direct visualization of recombinant His-tagged proteins, and other proteins bearing histidine tags in western or dot blotting applications.

GoldiBlot™ improves greatly upon standard detection by anti-6xHis antibodies:

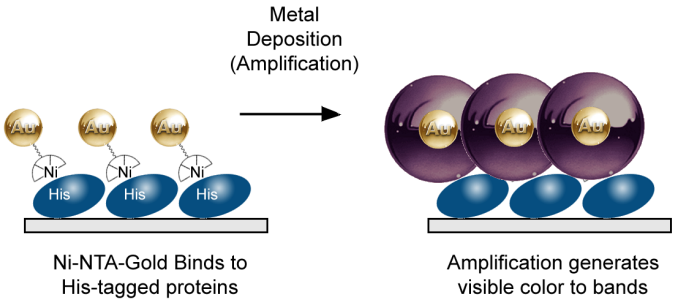
- Does not require a specific location of the polyhistidine tag (*N*- or *C*-terminus)
- No need for specific adjacent amino acid sequences
- No incubation time --No primary or secondary antibodies needed!

PRINCIPLE OF GoldiBlot™ HIS-TAG WESTERN BLOT KIT

GoldiBlot™ His-Tag Western Blot Kit uses Ni-NTA (nickel-nitrilotriacetic acid)-functionalized gold nanoparticles to specifically and directly bind to His-tagged proteins[1-6]. With 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-Tag Western Blot Kit can detect nanogram levels of purified His-tagged proteins, and detects His-tagged proteins in crude extract as well. The entire procedure takes about 1 hour.



Ni-NTA-Nanogold®, showing mechanism of binding to a polyhistidine (His) – tagged protein



Principle of GoldiBlot™: gold binding followed by autometallographic amplification (deposition of metal selectively onto the gold particles) generates visible signal.

REAGENTS PROVIDED

The following materials are sufficient for 15 mini-blots (7 cm x 8.4 cm) of membrane

GoldiBlot™ Nickel-NTA-Nanogold®	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

- **TBS-0.1%T**: 20 mM Tris, 0.15 M NaCl, pH7.6, 0.1% (w/v) Tween® -20
- 5 % (w/v) nonfat dry milk in TBS-0.1%T
- **TBS-0.6%T**: 20 mM Tris, 0.15 M NaCl, pH7.6, 0.6% (w/v) Tween® -20
- 1 % (w/v) nonfat dry milk in TBS-0.6%T
- 10 mM imidazole in TBS-0.6%T

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 cm x 8.4 cm blot. Volumes can be adjusted for staining multiple blots or for one different-sized blot.

All GoldiBlot™ reagents and other required materials should be equilibrated to room temperature prior to the western blotting procedure. All incubations of the GoldiBlot™ western blotting are performed at room temperature with shaking.

1. Transfer proteins 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 TBS-0.1%T for 3 min.
3. Block the membrane with 5 % (w/v) nonfat dry milk in TBS-0.1%T for 15 min.
4. Add 0.1 ml of GoldiBlot™ Nickel-NTA-Nanogold® to 10 ml of 1 % (w/v) nonfat dry milk in TBS-0.6%T. Vortex. Place the membrane in the solution, and incubate the blot for 30 min.
5. Wash the membrane two times with 15 ml of 10 mM imidazole in TBS-0.6%T for 2 min each.
6. Wash the membrane three times with 15 ml of deionized water for 3 min 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 min, 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 min, 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 min. 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 min each to terminate the autometallographic amplification.
   Note: The light purple-colored membrane background fades away as the membrane dries out.
9. Air-dry the membrane.

   Note: The concentration of NaCl and Tween 20 in binding and washes (used in GoldiBlot™ Nickel-NTA-Nanogold® binding and imidazole washes) can be slightly adjusted to achieve an optimized signal-to-noise ratio. Less NaCl and Tween20 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).