GoldiBlot™ His Western Blot Kit

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PRODUCT INFORMATION

Product Name: GoldiBlot™ His Western Blot Kit
Catalog Number: 2090-10BLOTS
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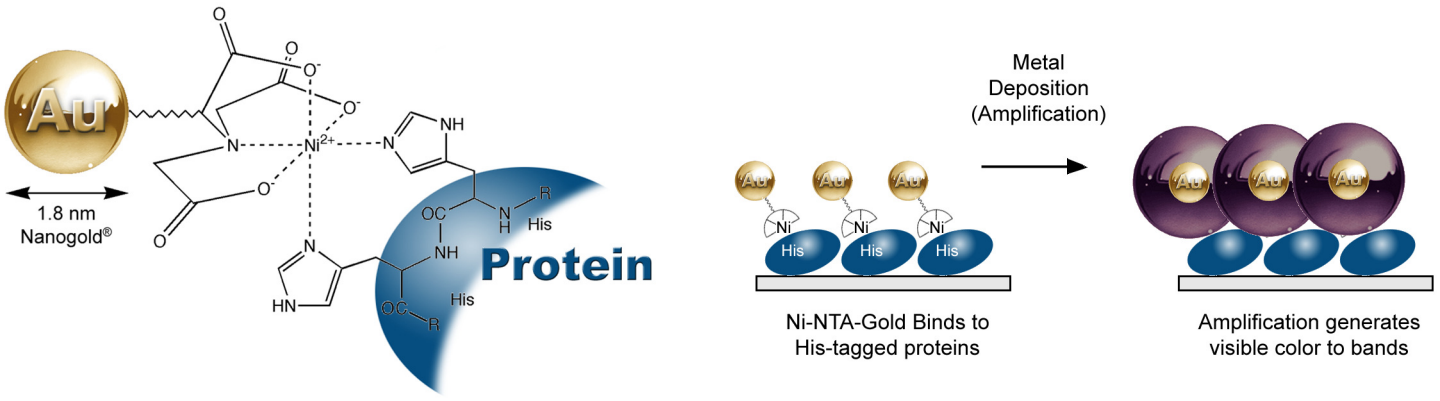
INTENDED USE

GoldiBlot™ His Western Blot Kit is intended and optimized for direct visualization of recombinant His-tagged proteins and other proteins bearing different polyhistidine (His) tags in western or dot blotting applications. Unlike detection by some anti-6xHis antibodies, GoldiBlot™ His detection does not require a specific location of the polyhistidine tag (*N*- or *C*-terminus) or the presence of specific adjacent amino acid sequences, and can recognize internal His-tags in addition to those at the *N*- or *C*-terminus. Probing is complete in one step, without the need for primary and secondary antibody incubations.

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]. When autometallographic amplification is 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

#2090-10BLOTS kit contains the following materials:

GoldiBlot™ Nickel-NTA-Au	1 mL
GoldiBlot™ AutoMet Detect A	27 mL
GoldiBlot™ AutoMet Detect B	27 mL
GoldiBlot™ AutoMet Detect C	27 mL
GoldiBlot™ AutoMet Detect D	27 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 upon receipt. 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 blot. Volumes may be adjusted for different sized or multiple blots.

All GoldiBlot™ reagents and other required materials should be equilibrated to room temperature prior to using for 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 or nitrocellulose membrane.
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. Bands loaded with more than 100 ng His-tagged protein may be seen within 6 minutes. Longer incubation times may be needed in order to see less than 20 ng His-tagged protein. However, longer incubation may lead to some non-specific background binding.
8. Wash the membrane three times for three minutes each time with 15 mL of deionized water to terminate autometallographic amplification.
   Note: Any light background color of the membrane back fades 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 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

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