GoldiBlot™ His Western Blot Kit

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 autometallographic 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 cm² 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 cm² 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 orbital 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.

*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


