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-nitritriacetic acid)-functionalized gold nanoparticles to specifically and directly bind to His-tagged proteins. 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.
REAGENTS PROVIDED

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
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<tbody>
<tr>
<td>GoldiBlot™ Nickel-NTA-Nanogold®</td>
<td>1.5 mL</td>
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<tr>
<td>GoldiBlot™ AutoMet Detect A</td>
<td>40 mL</td>
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<tr>
<td>GoldiBlot™ AutoMet Detect B</td>
<td>40 mL</td>
</tr>
<tr>
<td>GoldiBlot™ AutoMet Detect C</td>
<td>40 mL</td>
</tr>
<tr>
<td>GoldiBlot™ AutoMet Detect D</td>
<td>40 mL</td>
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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.

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