10 nm Ni-NTA-Nanogold®

Nanoprobes

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PRODUCT INFORMATION

Catalog Number:	2084
Product Name:	10 nm Ni-NTA-Nanogold®
Quantity:	3 mL (concentration 4.5 x 10^{-8} M)
Appearance:	Dark red solution
Revision:	1.0 (March 2017)
Storage:	Upon receipt store product at 2-8°C. Product is shipped at ambient temperature.

10 nm Ni-NTA-Nanogold®[1] can be used to detect or localize 6xHistidine (His) or Poly-His tagged recombinant proteins without the use of anti-His-tag antibodies in protein mixtures, multisubunit protein complexes, tissue or cell samples, using electron microscopy (EM), light microscopy, blots and plate-based assays. Using 10 nm Ni-NTA-Nanogold®, His-tagged recombinant proteins originating from a variety of expression vectors can be labeled under both non-denaturing and denaturing buffer conditions. The labeled His-tagged recombinant proteins can be detected by electron microscopy using low magnification, directly visualized as pink bands on western blots, or measured using a spectrophotometer in microplate assays without the use of gold or silver enhancement. 10 nm Ni-NTA-Nanogold® can also be used to identify His-tagged proteins containing eluent fractions from Immobilized Metal Affinity Chromatography (IMAC) procedures using a simple dot blot procedure.

CONTENTS

Warning: For research use only. Do not use internally or externally in humans or animals.

INTRODUCTION

The His-tag, consisting of five to ten consecutive histidine residues, has been used for detection and purification of proteins by immobilized metal-ion affinity chromatography (IMAC).[2,3] The use of a His tag provides several advantages. There is minimal addition of extra amino acids to the recombinant proteins. The small histidine tail is poorly immunogenic and usually does not interfere with protein folding. His-tagged proteins can have an extremely high affinity for metal ions including nickel, cobalt and copper, which allows the detection and purification of the His-tagged proteins using Ni-NTA (nickel (II) nitrilotriacetic acid) functionalities (K_a=10^{13} M).[3-13] 10 nm Ni-NTA-Nanogold® consists of a 10 nm gold particle with multiple Ni-NTA functionalities incorporated into ligands on the surface of gold particle. Each Ni^{2+} is complexed with one NTA, leaving two adjacent coordination sites which bind to two histidines from the His-tagged recombinant protein to form a stable complex (Figure 1). Strong binding is achieved when three adjacent Ni-NTA groups bind to a 6x-His tag. The His-tagged proteins are thus labeled, and the 10 nm gold particle may be used to localize the His-taged protein by electron microscopy or detect it on blots or using spectrophotometric readers without using gold or silver enhancement. Compared with immunogold labeling, the use of 1.8 nm, 5 nm and 10 nm Ni-NTA-Nanogold® provides the advantage of more precise localization of the His tag sites, since no additional protein entities or antibodies are involved and the distance from the gold particle surface to the His tag is less than 1.5 nm. 1.8 nm, 5 nm and 10 nm Ni-NTA-Nanogold® bind to N-terminal, C-terminal and internal His-tag sequences, and recognize from five to ten consecutive histidine residues encoded by a variety of commercial available expression vectors.

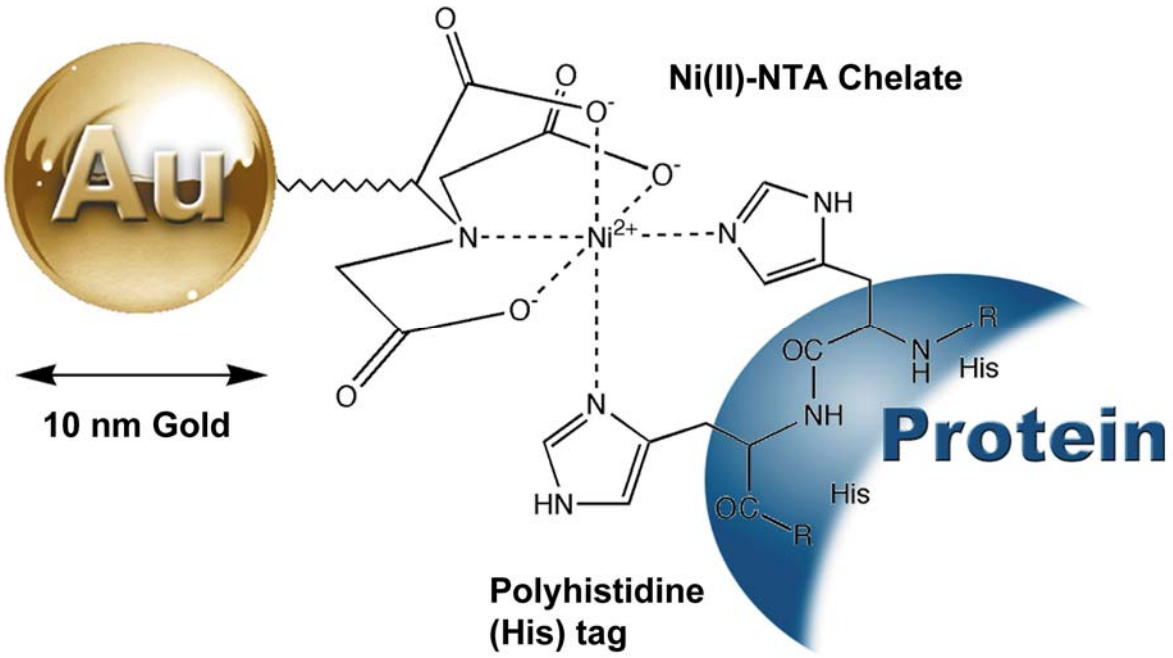

Figure 1: Interaction between a His-tagged protein and 10 nm Ni-NTA-Nanogold® (Catalog Number 2084).

PRODUCT INFORMATION

This product is supplied as a red colored liquid at a concentration of 4.5 x 10^{-8} M in 50 mM MOPS, pH 7.9. No additional stabilizer or preservative is included. If a sterile solution is needed, the product solution can be filtered with a 0.2 um cellulose acetate membrane filter. As supplied, this product is stable at least 1 year when stored at 2-8°C.

GENERAL CONSIDERATIONS FOR THE USE OF 10 nm Ni-NTA-NANOGOLD®

1. His-tagged protein samples should be prepared in a binding buffer at pH 7 – 8. While His tags are labeled with 10 nm Ni-NTA-Nanogold®, nonspecific binding can also occur as many proteins have intrinsic histidine and/or cysteine amino

acid residues. It is often necessary to include 5 - 20 mM imidazole to prevent nonspecific binding. Binding and washing conditions may be optimized by varying the concentrations of imidazole and sodium chloride in the binding and wash buffers. Increasing the concentration of imidazole and sodium chloride generally decreases nonspecific binding, but also weakens affinity interaction: it may be necessary to test some different combinations to find the right balance. 5 – 20 mM imidazole and 150 – 500 mM NaCl are generally found to give satisfactory labeling results. Including 1% (w/v) nonfat dry milk in the binding buffer and 0.1% (w/v) Tween-20 in the binding and wash buffers also helps reduce nonspecific binding in tissue and cell samples. Buffers with low pH (<5.0) protonate the histidines and disrupt the interaction with Ni (II), and therefore should not be used.

2. Binding and washing buffers must be free of thiols such as β-mercaptoethanol which can degrade gold particles, or reducing or chelating agents such as DTT, EDTA or citrate which may compete with His-tag or NTA binding. Samples containing EDTA, DTT, or citrate may give low specific staining.

3. His tagged protein samples should be incubated with 10 nm Ni-NTA-Nanogold®, diluted 1 : 5 – 1 : 100 in the binding buffer for 5 -30 minutes at room temperature. The optimum concentration of 10 nm Ni-NTA-Nanogold® to be used needs to be determined for each application. For EM study, His tagged protein complexes can be immobilized on a glow-discharged, carbon-coated electron microscopy grid prior to labeling. The grid can then be incubated upside-down on a droplet of 10 nm Ni-NTA-Nanogold® solution on parafilm. Protein complexes can also be labeled in solution. However, the gold-to-His ratio and concentrations must be carefully controlled to avoid the formation of aggregates, since each gold particle contains multiple Ni-NTA groups. Using a 5 to 10-fold molar excess of 10 nm Ni-NTA-Nanogold® in a diluted mixture can help reduce the formation of aggregates. After incubation, the excess 10 nm Ni-NTA-Nanogold® can be removed by gel filtration, ion exchange liquid chromatography, dialysis or centrifugation.

4. For applications involving tissues or cells, block the sample with 20 mM Tris, 150 mM NaCl, 0.1% Tween-20 at pH 7.6 containing 1 - 5% Nonfat dried milk for 5-30 minutes at room temperature prior to the application of 10 nm Ni-NTA-Nanogold®. This step helps block nonspecific protein binding sites, and minimizes nonspecific interactions.

5. Osmium tetroxide, uranyl acetate, lead acetate, tungsten- or vanadium-based negative stains may be used to contrast the edges of single particles. Tungsten (Nano-W™, catalog number 2018) or vanadium (NanoVan™, catalog number 2011) - based negative stains are especially useful for negatively staining small single particle structures such as multisubunit protein complexes. NanoVan™ gives a lighter stain than uranium, lead or tungsten-based negative stains, and allows easier visualization of smaller gold nanoparticles.[14]

EXAMPLE PROTOCOLS

Note. The following protocols are general examples of applications for this product. Specific experiments may require optimization.

Materials

1. TBS: 20 mM Tris at pH 7.6 with 150 mM NaCl
2. PBS: 20 mM sodium phosphate at pH 7.4 with 150 mM NaCl
3. PBS-Milk: 20 mM sodium phosphate at pH 7.4 with 150 mM NaCl containing 1 % (w/v) non-fat milk and 0.1% (w/v) cold fish gelatin (high purity). It has been found that nonfat milk and cold fish gelatin helps reduce background or nonspecific binding. Including 0.5 M NaCl and 0.05% (v/v) Tween-20 also helps reduce nonspecific binding.
4. PBS-BSA: 20 mM sodium phosphate at pH 7.4 with 150 mM NaCl containing 0.5 % (w/v) BSA and 0.1% (w/v) cold fish gelatin (high purity).
5. TBS-0.1%T: 20 mM Tris at pH 7.6 with 150 mM NaCl containing 0.1% (w/v) Tween-20

Labeling His-Tagged Protein Complexes in Solution

1. Prepare protein complex in 20 mM Tris at pH 7.6 with 150 mM NaCl.

2. Incubate protein complex with 10 molar excess of 10 nm Ni-NTA-Nanogold® for 30 min at room temperature or 4°C.
3. Remove the unbound gold nanoparticles from labeled protein conjugates using gel filtration, ion exchange chromatography, centrifugation or dialysis. Gel filtration chromatography media such as GE Healthcare Superose or Superdex or Bio-rad Bio-Gel are recommended: selection of a specific gel should be based on the molecular weight of the proteins and the preferred fractionation range. Concentrate the reaction mixture to a suitable volume for injection using membrane centrifugation (e.g. Amicon Ultra-4, Millipore). Elute with TBS. The first colored peak or shoulder is the conjugate, while the second dark colored band is the excess gold nanoparticles.
4. If desired, remove salt using membrane centrifugation or dialysis.
5. Load gold labeled protein complex on a carbon coated EM grid.
6. Apply negative stain such as NanoVan™, (catalog number 2011) according to product instructions before examination.

Labeling His-Tagged Protein Complexes on Grids

1. Load purified His-tagged protein complex on glow-discharged carbon coated EM grids, and remove excess liquid using filter paper.
2. Place grid upside-down on a droplet of 10 nm Ni-NTA-Nanogold® diluted 1/30 and incubate for 30 min at room temperature.
3. If desired, wash the grid upside-down on a droplet of 20 mM Tris at pH 7.6 with 150 mM NaCl containing 8 mM imidazole for 1 min at room temperature.
4. Rinse with deionized water.
5. Perform negative stain such as NanoVan™, (catalog number 2011) according to product instructions before examination.

Pre-Embedding Labeling of His-Tagged Proteins in Tissue/Cell Samples

Labeling tissues or cells before embedding and sectioning (the pre-embedding method)[15,16] is used for the study of surface targets. It gives good preservation of cellular structure, and subsequent staining usually produces high contrast for study of the cellular details.

Cells in Suspension

1. Optional fixing of cells: e.g., with glutaraldehyde (0.05 - 1% for 15 minutes) in PBS. Do not use Tris buffer since this contains an amine. After fixation, centrifuge cells (e.g. 1 mL at 10^7 cells/mL) at 300 X g, 5 minutes; discard supernatant; resuspend in 1 mL PBS. Repeat this washing (centrifugation and resuspension) 2 times.
2. Incubate cells with 0.02 M glycine in PBS (5 mins) to quench the remaining aldehyde. Centrifuge, then resuspend cells in PBS-BSA buffer (specified above) for 5 minutes.
3. Wash cells with PBS-BSA using repeated centrifugation and resuspension as described in step 1 (2 X 5 mins). Resuspend in 1 mL PBS-BSA.
4. Place 50-200 µL of cells into Eppendorf tube. Dilute 10 nm Ni-NTA-Nanogold® (1/20) in PBS-Milk buffer and add 30-50 µL to cells; incubate for 15-30 minutes with occasional shaking.
5. Wash cells in PBS-BSA by repeated centrifugation and resuspension as described in step 1 (2 X 5 mins). 5-20 mM imidazole may be included if nonspecific binding is concerned.
6. Fix cells using a final concentration of 1% (v/v) glutaraldehyde in PBS for 15 minutes. Then remove fixative by repeated centrifugation and resuspension with PBS (3 X 5 mins).
7. Rinse in deionized water (2 X 5 min).
8. Dehydrate and embed according to usual procedure. Use of a low-temperature resin (e.g. Lowicryl) is recommended.
9. Stain with uranyl acetate, or lead citrate or other staining reagent before examination

Tissue Sections

1. Float on a drop of water for 5 - 10 minutes.

2. Incubate with PBS-Milk for 5 minutes to block nonspecific protein binding
3. Rinse with PBS-Milk (1 min).
4. Incubate with 10 nm Ni-NTA-Nanogold® diluted 1/5 – 1/100 in PBS-BSA for 15-30 minutes at room temperature.
5. Rinse with PBS containing 5 mM imidazole (1 min), then PBS (3 X 1 min).
6. Postfix with 1% glutaraldehyde in PBS (10 mins).
7. Rinse in deionized water (2 X 5 min).
8. Dehydrate and embed according to usual procedure. Use of a low-temperature resin (e.g. Lowicryl) is recommended.
9. Stain with uranyl acetate, or lead citrate or other staining reagent before examination.

Post-Embedding Labeling of His-Tagged Proteins in Tissue/Cell Samples

Labeling after embedding and sectioning (the post-embedding method)[15,16] allows the access of Ni-NTA-Nanogold® to the interior of the cells or tissues, and is used to label both exterior and interior targets.

Note. Thin sections mounted on grids are floated on drops of solutions on parafilm or in well plates. Hydrophobic resins usually require pre-etching.

1. Prepare sections on plastic or carbon-coated nickel grid. Float on a drop of water for 5 - 10 minutes.
2. Incubate with PBS-Milk for 5 minutes to block non-specific protein binding sites.
3. Rinse with PBS-Milk (1 min).
4. Incubate with 10 nm Ni-NTA-Nanogold® diluted 1/5 – 1/100 in PBS-Milk for 15-30 minutes at room temperature.
5. Rinse with PBS containing 5 mM imidazole (1 min), then PBS (3 X 1 min).
6. Postfix with 1% glutaraldehyde in PBS at room temperature (3 mins).
7. Rinse in deionized water for (2 X 5 min).
8. Stain with uranyl acetate, or lead citrate or other positive staining reagent before examination

Procedure for Detecting His-Tagged Proteins on Western Blots

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

1. Transfer proteins from gel to a nitrocellulose or PVDF 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 0.5 ml of 10 nm Ni-NTA-Nanogold® to 10 ml of 50 mM MOPs, pH 7.9. Vortex. Place the membrane in the solution, and incubate the blot for 30 min.
5. Wash the membrane one time with 15 ml of 10 mM imidazole in TBS-0.1%T for 2 min.
6. Wash the membrane two times with 15 ml of TBS-0.1%T for 3 min each
7. Wash the membrane three times with 15 ml of deionized water for 3 min each.
8. Air-dry the membrane.

   Note: The bands loaded with more than 50 ng His-tagged proteins can be seen as pink bands. Longer incubation time may be needed in order to see less than 50 ng but higher than 10 ng His-tagged proteins. An autometallographic amplification reagent e.g. GoldEnhance™ Blots (Catalog No. 2115) can be used after Step 7 to detect at least 1 ng His-tagged proteins

Procedure to Identify His-Tagged Protein Containing Eluent Fractions from IMAC

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

1. Blot 1-2 µL of eluent fractions from immobilized metal-ion affinity chromatography (IMAC) onto a nitrocellulose or PVDF membrane. Mark the fraction numbers on membrane.
2. Air dry for 15 minutes.
3. Block the membrane with 5 % (w/v) nonfat dried milk in TBS-0.1%T for 15 minutes.
4. Dilute 10 nm Ni-NTA-Nanogold® 1/20 with 50 mM MOPS, pH 7.9. Vortex. Place the membrane in the solution, and incubate the blot for 30 minutes.
5. Wash the membrane one time with 10 mM imidazole in TBS-0.1%T for 2 minutes.
6. Wash the membrane two times with TBS-0.1%T for 3 minutes each
7. Wash the membrane three times with deionized water for 3 minutes each.
8. Air-dry the membrane for permanent record.

Note: Dots containing more than 50 ng His-tagged proteins can be seen as pink colored spots. Longer incubation time may be needed in order to see less than 50 ng but higher than 10 ng His-tagged proteins. An autometallographic amplification reagent e.g. GoldEnhance™ Blots (Catalog No. 2115) may be used after Step 7 for more sensitive detection of at least 1 ng of His-tagged protein.

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Technical Assistance Available.

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