NANOGOLD®

Nanoprobes

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PRODUCT INFORMATION

MONO-ALKYNE-NANOGOLD® LABELING REAGENT

Product Name:	Mono-Alkyne-Nanogold®
Catalog Number:	2027-30 nmol
	2027-5x6 nmol
	2027-6 nmol
Appearance:	Brown Powder/Solid
Revision:	1.1 (August 2019)

Congratulations on your acquisition of a revolutionary new gold labeling reagent: Mono-Alkyne-Nanogold®. With this reagent you can label your azide or any azide-containing molecule of interest, including peptides, proteins, oligonucleotides, and cellular components, with Nanogold® particle for localization and detection.

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Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. Non radioactive and non carcinogenic.

PRODUCT INFORMATION

Nanogold® is a specially developed gold label, prepared using a discrete gold compound rather than a colloid.[1] This kit contains the Nanogold® particle with an alkyne functionality incorporated into a ligand on the surface of the gold particle; this specifically reacts with an azide to form a 1,2,3-triazole in the 1,3-dipolar cycloaddition catalyzed by copper, Cu(I)[2,3] (Figure 1). The advantage of the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction is that it is biorthogonal: azides and alkynes react selectively only with each other, and not with any naturally occurring cellular components. The copper, Cu(I), is generated in situ from Cu(II) with the use of an reducing agent, or sodium ascorbate in the presence of accelerating ligands, e.g. tris(3-hydroxypropyltriazolymethyl)amine, THPTA[4]. The water-soluble THPTA click ligand binds Cu(I), protects against histidine oxidation, and intercepts reactive oxygen species, affording biological compatibility for Click reactions[5]. The THPTA ligand was effectively used to label live cells with high efficiency while maintaining cell viability[6].

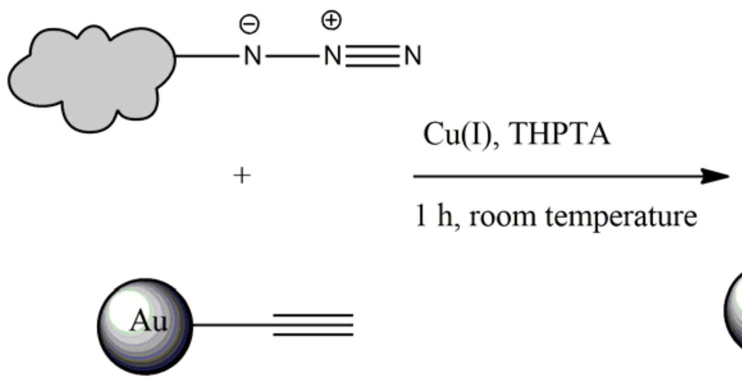

Figure 1: Schematic showing mono-alkyne-Nanogold® labeling of an azide via 1, 3-dipolar cycloaddition catalyzed by copper, Cu(I)

Mono-Alkyne-Nanogold® reagent as supplied has been lyophilized from 0.1 M sodium phosphate at pH 7.0. The solid labeling reagent should be stored at -20°C. Dissolution in 0.5 mL of deionized water will produce a solution of activated Nanogold® in 0.1 M sodium phosphate at pH 7.0 for Click conjugations. Nanogold® conjugates are stable to wide ranges of pH and ionic strength, and are not radioactive or carcinogenic. Nanogold®-labeled biomolecules prepared via CuAAC reactions can be detected or localized by electron microscopy, or light microscopy as well as on gels and blots using silver or gold enhancement: Nanogold® will nucleate silver or gold metal deposition, resulting in a dense particle 2 - 80 nm in size or larger depending on development time. Detailed enhancement instructions can be found with each enhancer kit.

Silver and gold enhancement kits:

2012-45 mL	HQ Silver™ Best for EM: Uniform enhancement, low background and excellent ultrastructural preservation.
2013-250 mL	LI Silver™ Use to stain Nanogold® labeled proteins or nucleic acids for light microscopic observation, in gels and on blots.
2112-28 mL	GoldEnhance™ LM Brown colored stains. High sensitivity and low background.
2113-8 mL	GoldEnhance™ EM High sensitivity and rapid enhancement.
2114-8 mL	GoldEnhance™ EM Plus Uniform enhancement and high sensitivity.
2115-48 mL	GoldEnhance™ Blots Purple colored stain. High sensitivity and rapid enhancement for direct optical and visual detection.

For more information, visit:
http://www.nanoprobes.com/products/Silver-Enhancers.html (silver enhancement)
http://www.nanoprobes.com/products/GoldEnhance.html (gold enhancement)

THIOL CAUTION

Nanogold® particles degrade upon exposure to thiols such as ß-mercaptoethanol or dithiothreitol. Thiol compounds used in the reduction of protein molecules (or other biomolecules) should be removed from the protein by gel filtration before Nanogold® conjugation. Dialysis does NOT provide acceptable purification in this application. A small amount of residual thiol reagent can severely limit the performance of Nanogold®.

OTHER CAUTIONS

Although Nanogold® is usually stable under demanding conditions, including pH values lower than 4 or ionic strengths above 1 M, Nanogold® reagents labeled specimens or conjugates may not be stable above 50°C for extended period of time, .e.g. over one week, and best results are obtained at room temperature or 4°C. In such cases, incubations at 37°C for extended period of time shall be avoided, and the use of low temperature embedding media (e.g., Lowicryl) is recommended if labeling before embedding[7]. It is not recommended to bake tissue blocks with Nanogold®. If your experiment requires higher temperature embedding, then silver enhancement should be performed before embedding.

CLICK REACTION CONDITIONS

EXAMPLE PROTOCOL

This section contains a general protocol for click reactions. This protocol may be used as a starting point for optimization of your particular click chemistry procedures.

Procedure using 30 nmol size (catalog # 2027-30 nmol):

1. Prepare the following click solutions:
   - 15 mM THPTA ligand in water
   - 6 mM $CuSO_4$ in water
   - 500 µM azide-modified molecule to be labeled in 0.1 M phosphate pH 7.0 containing 10% DMSO
   - 300 mM sodium ascorbate in water

2. Mix 10 µL of 6 mM $CuSO_4$ with 20 µL of 15 mM THPTA in a conical tube.

3. Add 0.5 mL deionized water to one vial of 30 nmol Mono-Alkyne-Nanogold®. Vortex. It will yield 0.5 mL of 60 µM Mono-Alkyne-Nanogold® in 0.1 M phosphate pH 7.0.

4. Add 60 µL of 500 µM azide-modified molecule to be labeled to 0.5 mL of Mono-Alkyne-Nanogold® solution. Vortex.

5. Add the mixture of azide and Mono-Alkyne-Nanogold® to the mixture of $CuSO_4$ and THPTA.

6. Add 10 µL of 300 mM sodium ascorbate to the mxiture from Step 5. Vortex. Incubate on a shaker at room temperature for 1 hour.

7. Purify Nanogold® conjugated peptide or proteins from unlabeled peptide or protein or excess Nanogold® reagents using gel filtration chromatography, e.g. Biorad P-30, or perform ethanol-precipitation on oligo conjugates. The resulting conjugated proteins in lysate are ready for downstream processing or analysis.

Procedure using 6 nmol size (catalog # 2026-5 x 6 nmol or 2026-6 nmol):

1. Prepare the following click solutions:
   - 15 mM THPTA ligand in water
   - 6 mM $CuSO_4$ in water
   - 500 µM azide-modified molecule to be labeled in 0.1 M phosphate pH 7.0 containing 10% DMSO
   - 300 mM sodium ascorbate in water
2. Mix 2 µL of 6 mM $CuSO_4$ with 4 µL of 15 mM THPTA in a conical tube.
3. Add 0.1 mL deionized water to one vial of 6 nmol Mono-Alkyne-Nanogold®. Vortex. This will yield 0.1 mL of 60 µM Mono-Alkyne-Nanogold® in 0.1 M phosphate pH 7.0.
4. Add 12 µL of 500 µM azide-modified molecule to be labeled to 0.1 mL of Mono-Alkyne-Nanogold® solution. Vortex.
5. Add the mixture of azide and Mono-Alkyne-Nanogold® to the mixture of $CuSO_4$ and THPTA.
6. Add 2 µL of 300 mM sodium ascorbate to the mxiture from Step 5. Vortex. Incubate on a shaker at room temperature for 1 hour.
7. Purify Nanogold® conjugated peptide or proteins from unlabeled peptide or protein or excess Nanogold® reagents using gel filtration chromatography, e.g. Biorad P-30, or perform ethanol-precipitation on oligo conjugates. The resulting conjugated proteins in lysate are ready for downstream processing or analysis.

In addition to direct labeling of purified alkyne-modified molecules or lysates, the biorthogonal nature of the Click reaction makes it well suited to labeling in cells, tissues and even in vivo. See Hong et al[6] for detailed protocol and suggestions for live cell labeling.

CHARACTERIZATION OF NANOGOLD® CONJUGATES

The purified Nanogold® conjugated peptide, or protein or polynucleotide is normally brown colored at a high concentration, and can be characterized by UV-Vis spectroscopy in the range of 250 nm - 800 nm. Unlike the UV-Vis spectrum of an unlabeled peptide, or protein or oligonucleotide, which is usually near or at baseline from 300 - 800 nm, the absorption spectrum of Nanogold®-conjugated peptide protein or oligonucleotide descends steadily over the range of 300 nm - 800 nm. When the molar extinction coefficient of the protein at 280 nm or oligonucleotide at 260 nm is 1.5×10^5 $M^{-1}cm^{-1}$ or greater, the Degree of Labeling (DOL) or the Nanogold®/protein or oligonucleotide molar ratio can be estimated using the absorbance at 280 nm or 260 nm, and 420 nm.

Estimation of Degree of Labeling (DOL): Dilute a portion of the purified Nanogold® conjugated protein or oligonucleotide so that the maximum absorbance at 280 nm (proteins) or 260 nm (oligonucleotides) is 0.7 to 1.2 AU. Measure the absorbance at 280 nm (proteins) or 260 nm (oligonucleotides) and 420 nm. Use the absorbance at 420 nm to calculate the molar concentration of the Nanogold® using the molar extinction coefficient of Nanogold® (155,000 $M^{-1}cm^{-1}$ at 420 nm). The molar concentration of the protein or polynucleotide can be calculated using A_{280nm} or A_{260nm} after subtracting the absorption due to Nanogold®, calculated using the values for $\gamma_{gold\ (280\ nm/420nm)}$ or $\gamma_{gold\ (260\ nm/420nm)}$ provided in the product specification sheet.

$$[\text{Nanogold}^{®}] = [A_{420nm}]/155{,}000$$

$$[\text{Protein}] = [A_{280nm} - \gamma_{gold,\ 280\ nm/420nm} \times A_{420nm}]/\varepsilon_{protein\ at\ 280\ nm}$$

or

$$[\text{Nucleic Acid}] = [A_{260nm} - \gamma_{gold,\ 260\ nm/420nm} \times A_{420nm}]/\varepsilon_{oligonucleotide\ at\ 260\ nm}$$

DOL = [Nanogold®]/[Protein]

or

DOL = [Nanogold®]/[oligonucleotide]

Characterization by Gel Electrophoresis: Purified Nanogold® conjugates or Nanogold® conjugate mixtures can also be characterized using SDS gel, native gel or agarose gel. For best results, follow the procedure below:

1. Use a gel with two panels or lanes. Load purified Nanogold® conjugate, or Nanogold® conjugate mixture with unlabeled peptide, protein or oligonucleotide and unreacted Nanogold® reagent into the left panel of the gel.

2. Duplicate the loading in the same sequence and amounts into the right panel.

3. Run the gel to reach separation. Nanogold® has a molecular weight of about 15,000 dalton and negligible charge, and contributes little to the charges of labeled peptides, proteins or polynucleotides. **Caution**: Nanogold® conjugates and Nanogold® reagents should not be heated with ß-mercaptoethanol before loading onto gels as ß-mercaptoethanol degrades Nanogold® particles during incubation.

4. After running the gel to reach separation, cut the gel in the middle to separate the two lanes.

5. Wash one panel with deionized water for 3 x 15 minutes, then incubate this panel with LI silver™ (Nanoprobes Catalog #2013-250 mL) for 10 minutes. Wash with deionized water for 4 x 5 minutes and continue overnight. The Nanogold® conjugate and Nanogold® reagent bands will become brown in color upon incubation with LI Silver™.

6. The other panel should be stained either with Coomassie stain (for proteins) or nucleic acid stains (for nucleic acids). Nanogold® conjugates with these molecules and unlabeled peptide, protein or nucleic acid will be stained.

Nanogold® conjugate bands will be stained by both LI Silver™ and Coomassie or nucleic acid stains.

GENERAL CONSIDERATIONS FOR USING MONO-ALKYNE NANOGOLD® REAGENTS

- Nanogold® is an extremely uniform 1.4 nm diameter gold particle (±10%).

- Mono-Alkyne-Nanogold® reacts with an azide to form a 1,2,3-triazole conjugate in CuAAC.

- Nanogold® is covalently attached to the peptide, protein, oligonucleotide or live cells after click reactions.

- Nanogold® conjugates contain no aggregates. This is in sharp contrast to other colloidal gold conjugates that are usually prepared by centrifugation to remove the largest aggregates and frequently contain smaller aggregates.

- Nanogold® particles do not have affinity to proteins as do colloidal golds. This reduces background and false labeling.

- Nanogold® develops better with silver than do most other colloidal golds giving it higher sensitivity. Both silver and gold enhancement can be used to enlarge Nanogold® to desirable sizes for electron and light microscopy, gel and blot detection.

USING STAINS WITH NANOGOLD®

Because the 1.4 nm Nanogold® particles are so small, over staining with OsO_4, uranyl acetate or lead citrate may tend to obscure direct visualization of individual Nanogold® particles. Three recommendations for improved visibility of Nanogold® are:

1. Use of reduced amounts or concentrations of usual stains.
2. Use of lower atomic number stains such as Nanoprobes NanoVan™, a Vanadium based stain.[8]
3. Enhancement of Nanogold® with silver or gold enhancers.

SPECIAL CONSIDERATIONS FOR DIRECT VIEWING OF NANOGOLD® IN THE ELECTRON MICROSCOPE

For most work, silver or gold enhancement is recommended to give a good signal in the electron microscope (see below). For particular applications, visualization of the Nanogold® directly may be desirable. Generally this requires very thin samples and precludes the use of other stains.

Nanogold® provides a much improved resolution and smaller probe size over other colloidal gold antibody products. However, because Nanogold® is only 1.4 nm in diameter, it will not only be smaller, but will appear less intense than, for example, a 5 nm gold particle. With careful work, however, Nanogold® may be seen directly through the binoculars of a standard EM even in 80 nm thin sections. However, achieving the high resolution necessary for this work may require new demands on your equipment and technique. Several suggestions follow:

1. Before you start a project with Nanogold® it is helpful to see it so you know what to look for. Dilute the Nanogold® stock 1:5 and apply 4 µl to a grid for 1 minute. Wick the drop and wash with deionized water 4 times.

2. View Nanogold® at 100,000 X magnification with 10 X binoculars for a final magnification of 1,000,000 X. Turn the emission up full and adjust the condenser for maximum illumination.

3. The alignment of the microscope should be in order to give 0.3 nm resolution. Although the scope should be well aligned, you may be able to skip this step if you do step 4.

4. Objective stigmators <u>must</u> be optimally set at 100,000 X. Even if the rest of the microscope optics are not perfectly aligned, adjustment of the objective stigmators may compensate and give the required resolution. You may want to follow your local protocol for this alignment but since it is important, a brief protocol is given here:

   a. At 100,000 X (1×10^6 with binoculars), over focus, under focus, then set the objective lens to <u>in focus.</u> This is where there is the least amount of detail seen.

   b. Adjust each objective stigmator to give the least amount of detail in the image.

   c. Repeat steps a and b until the in focus image contains virtually no contrast, no wormy details, and gives a flat featureless image.

5. Now underfocus slightly, move to a fresh area, and you should see small black dots of 1.4 nm size. This is the Nanogold®. For the 1:5 dilution suggested, there should be about 5 to 10 gold spots on the small viewing screen used with the binoculars. Contrast and visibility of the gold clusters is best at 0.2 - 0.5 m defocus, and is much worse at typical defocus values of 1.5 - 2.0 m commonly used for protein molecular imaging.

6. In order to operate at high magnification with high beam current, thin carbon film over fenestrated holey film is recommended. Alternatively, thin carbon or 0.2% Formvar over a 1000 mesh grid is acceptable. Many plastic supports are unstable under these conditions of high magnification/high beam current and carbon is therefore preferred. Contrast is best using thinner films and thinner sections.

7. Once you have seen Nanogold® you may now be able to reduce the beam current and obtain better images on film. For direct viewing with the binoculars, reduction in magnification from 1,000,000 X to 50,000 X makes the Nanogold® much more difficult to observe and not all of the golds are discernable. At 30,000 X (300,000 X with 10 X binoculars) Nanogold® particles are not visible. It is recommended to view at 1,000,000 X, with maximum beam current, align the objective stigmators, and then move to a fresh area, reduce the beam, and record on film.

8. If the demands of high resolution are too taxing or your sample has an interfering stain, a very good result may be obtained using silver or gold enhancement to give particles easily seen at lower magnification.

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