UNDECAGOLD

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PRODUCT INFORMATION

MONO-AZIDO-UNDECAGOLD LABELING REAGENT

Product Name:	Mono-Azido-Undecagold
Catalog Number:	2046-50 nmol
	2046-5x10 nmol
	2046-10 nmol
Appearance:	Orange Powder/Solid
Revision:	1.1 (August 2019)

Congratulations on your acquisition of a revolutionary new gold labeling reagent: Mono-Azido-Undecagold. With this reagent you can label your alkyne or any alkyne-containing molecule of interest, including peptides, proteins, oligonucleotides, and cells, with Undecagold particles for detection or localization. It is the smallest gold probe commercially available, with a diameter of just 0.8 nm.

CONTENTS

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

Undecagold is the smallest gold label available, prepared using a discrete gold compound rather than a colloid.[1] This kit contains the Undecagold particle with an azido functionality incorporated into a ligand on the surface of the gold particle; this specifically reacts with an alkyne to form a 1,2,3-triazole adduct 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 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]

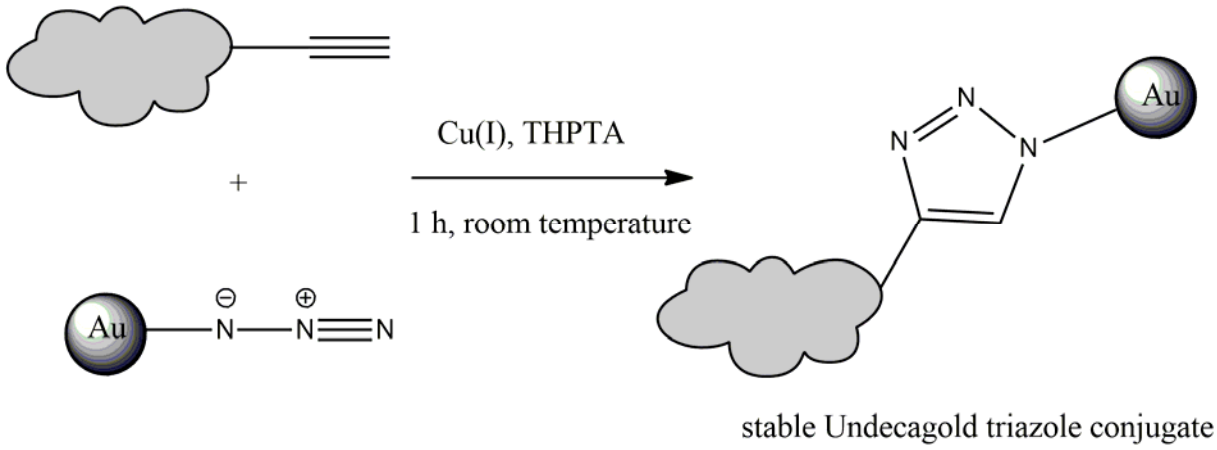

Figure 1: Schematic showing Mono-Azido-Undecagold labeling of an alkyne via 1,3-dipolar cycloaddition catalyzed by copper, Cu(I).

Mono-Azido-Undecagold reagent as supplied has been lyophilized from 0.1 M sodium phosphate at pH 7.0 containing 10% isopropanol. The solid labeling reagent should be stored at -20°C. Dissolution in 10% isopropanol will produce a solution of Mono-Azido-Undecagold for click conjugations. Undecagold conjugates are intended for use in high resolution electron microscopy where the smallest possible gold probe and the lowest possible interference with the bio-activity of the conjugate are desired. Undecagold conjugates are stable to wide ranges of pH and ionic strength, and are not radioactive or carcinogenic.

THIOL CAUTION

Undecagold 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 Undecagold conjugation. Dialysis does NOT provide acceptable purification in this application. A small amount of residual thiol reagent can severely limit the performance of Undecagold .

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 50 nmol size (catalog # 2046-50 nmol)

1. Prepare the following click solutions:
   - 15 mM THPTA ligand in water
   - 6 mM $CuSO_4$ in water
   - 833 µM alkyne-modified compound 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 of 10% isopropanol to one vial of 50 nmol Mono-Azido-Undecagold. Vortex. This will yield 0.5 mL of 100 µM Mono-Azido-Undecagold in 0.1 M phosphate pH7.0 containing 10% isopropanol.
4. Add 60 µL of 833 µM alkyne-modified compound to be labeled to 0.5 mL of Mono-Azido-Undecagold solution. Vortex.
5. Add the mixture of alkyne and Mono-Azido-Undecagold to the mixture of $CuSO_4$ and THPTA.
6. Add 10 µL of 300 mM sodium ascorbate to the mixture from Step 5. Vortex. Incubate on a shaker at room temperature for 1 hour.
7. Purify Undecagold-conjugated peptides or proteins from excess click reagents or unlabeled peptides or proteins using gel filtration chromatography, e.g. Biorad P-30, or perform ethanol-precipitation on oligonucleotide conjugates. The resulting conjugated proteins, peptides or oligonucleotides in lysate are ready for downstream processing or analysis.

Procedure using 10 nmol size (catalog # 2046-5 x 10 nmol or 2046-10 nmol)

1. Prepare the following click solutions:
   - 15 mM THPTA ligand in water
   - 6 mM $CuSO_4$ in water
   - 833 µM alkyne-modified compound 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 of 10% isopropanol to one vial of 10 nmol Mono-Azido-Undecagold. Vortex. This will yield 0.1 mL of 100 µM Mono-Azido-Undecagold in 0.1 M phosphate pH7.0 containing 10% isopropanol.
4. Add 12 µL of 833 µM alkyne-modified compound to be labeled to 0.1 mL of Mono-Azido-Undecagold solution. Vortex.
5. Add the mixture of alkyne and Mono-Azido-Undecagold to the mixture of $CuSO_4$ and THPTA.
6. Add 2 µL of 300 mM sodium ascorbate to the mixture from Step 5. Vortex. Incubate on a shaker at room temperature for 1 hour.
7. Purify Undecagold-conjugated peptides or proteins from excess click reagents or unlabeled peptides or proteins using gel filtration chromatography, e.g. Biorad P-30, or perform ethanol-precipitation on oligonucleotide conjugates. The resulting conjugated proteins, peptides or oligonucleotides 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.

Copper-free Click reactions: In addition to the copper-catalyzed procedure given above, Mono-Azido Undecagold may also be used in copper-free click reactions where azides are used as one of the reagents. In these cases, we recommend that you start by following

the protocol for the existing reaction, but substitute Mono-Azido Undecagold for the original azide, and adjust concentration, stoichiometric ratio, and conditions as necessary.

CHARACTERIZATION OF UNDECAGOLD CONJUGATES

The purified Undecagold conjugates of peptides, proteins or oligonucleotides are normally orange colored at high concentrations, and can be characterized by UV-Vis spectroscopy in the range 250 nm - 800 nm. Unlike the UV-Vis spectrum of an unlabeled peptide, protein or oligonucleotide, which is close to baseline in the 300 nm - 800 nm range, the absorption spectrum of Undecagold-conjugated peptide, protein or oligonucleotide descends over the range of 300 nm - 800 nm with shoulders at 310 nm and 420 nm. The Degree of Labeling (DOL), or the Undecagold / protein, peptide or oligonucleotide molar ratio, can be estimated using the absorbances at 280 nm (for proteins or peptides) or 260 nm (for oligonucleotides), and 420 nm.

Estimation of Degree of Labeling (DOL): Dilute a portion of the purified Undecagold-conjugated protein, peptide or oligonucleotide so that the maximum absorbance at 280 nm (for peptides or proteins) or 260 nm (for oligonucleotides) is 0.7 to 1.2 AU. Measure the absorbances at 280 nm (proteins or peptides) or 260 nm (oligonucleotides) and 420 nm. Use the absorbance at 420 nm to calculate the molar concentration of the Undecagold using the molar extinction coefficient of Undecagold (47,100 $M^{-1}cm^{-1}$ at 420 nm). The molar concentration of the protein or oligonucleotide can be calculated using A_{280nm} or A_{260nm}, after subtracting the absorption due to Undecagold, calculated using $\gamma_{\text{gold, 280 nm/420nm}}$ or $\gamma_{\text{gold, 260 nm/420nm}}$ provided in the product specification sheet.

$$[\text{Undecagold}] = [A_{420nm}]/47{,}100$$

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

or

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

$$\text{DOL} = [\text{Undecagold}]/[\text{Protein}]$$

or

$$\text{DOL} = [\text{Undecagold}]/[\text{oligonucleotide}]$$

Characterization by Gel Electrophoresis: Purified Undecagold conjugates or Undecagold 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 Undecagold conjugate, or Undecagold conjugate mixture with unlabeled peptide, protein or oligonucleotide and unreacted Undecagold 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. Undecagold has a molecular weight of about 5,400 dalton and negligible charge, and contributes little to the charges of labeled peptides, proteins or polynucleotides. **Caution**: Undecagold conjugates and Undecagold reagents should not be heated with ß-mercaptoethanol before loading onto gels as ß-mercaptoethanol degrades Undecagold 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 Undecagold conjugate and Undecagold 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). Undecagold conjugates with these molecules and unlabeled peptide, protein or nucleic acid will be stained.

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

GENERAL CONSIDERATIONS WITH UNDECAGOLD REAGENTS

- Undecagold is an extremely uniform 0.8 nm diameter gold particle (±10%).
- Mono-Azido-Undecagold reacts with an alkyne to form a 1,2,3-triazole adduct in CuAAC.
- Undecagold is covalently attached to the peptide, protein, oligonucleotide or live cells after click reactions
- Undecagold 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.
- Undecagold particles do not have affinity to proteins as do colloidal golds. This reduces background and false labeling.
- Undecagold can be enlarged using both silver and gold enhancers to desirable sizes for electron and light microscopy, gel and blot detection.

USING STAINS WITH UNDECAGOLD

Because the 0.8 nm Undecagold particles are so small, over staining with OsO_4, uranyl acetate or lead citrate will obscure direct visualization of individual Undecagold particles, and therefore these stains should not be used. Only light staining with a low atomic number stain, such as NanoVan™, a Vanadium based negative stain,[7] should be used.

SPECIAL CONSIDERATIONS FOR VIEWING UNDECAGOLD IN THE ELECTRON MICROSCOPE

Undecagold is the smallest gold probe commercially available, being just 0.8 nm in diameter. A high resolution instrument such as a Scanning Transmission Electron Microscope (STEM) is required for visualization; in a conventional TEM the Undecagold particles are not visible, and for TEM applications we usually recommend the larger and more easily visualized Nanogold®. With careful work, Undecagold may be seen directly in the STEM. 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 Undecagold it is helpful to see it so you know what to look for. Dilute the Undecagold stock 1:5 in methanol and apply 4 µL to a grid for 1 minute. Allow to dry.
2. View Undecagold using a full width scan of 128 nm or less; this will give sufficient magnification for visualization.
3. Undecagold is sensitive to beam damage (contrary to Nanogold® which is very beam-resistant); the behavior of Undecagold in the STEM has been described in the literature.[8] Image at approximately 200 $eÅ^{-2}$.
4. In order to operate at high magnification, thin carbon film over fenestrated holey film is recommended. 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.

SILVER ENHANCEMENT OF UNDECAGOLD FOR EM

Undecagold will nucleate silver or gold deposition resulting in a dense particle 2-20 nm in size or larger depending on development time. If specimens are to be embedded, silver or gold enhancement is usually performed after embedding, although it may be done first. It must be completed before any staining reagents such as osmium tetroxide, lead citrate or uranyl acetate are applied, since these will nucleate silver or gold deposition in the same manner as gold nanoparticles and produce non-specific staining.

Our silver and gold enhancement systems are convenient and suitable for all applications. Improved results in the EM may be obtained using HQ Silver™, which is formulated to give slower, more controllable particle growth and uniform particle size distribution with low background.

Specimens must be thoroughly rinsed with deionized water before silver or gold enhancement reagents are applied. This is because the buffers used for antibody incubations and washes contain chloride ions and other anions which form insoluble precipitates with silver, and the retaining buffers may alter the enhancement pH and performance. Follow specific instructions to prepare each develop before use.

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Technical Assistance Available.

For a complete list of references citing this product, please visit our web site at http://www.nanoprobes.com/Ref.html.