UNDECAGOLD

Nanoprobes

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PRODUCT INFORMATION

MONO-DBCO-UNDECAGOLD LABELING REAGENT

Product Name:	Mono-DBCO-Undecagold
Catalog Number:	2048-50NMOL
	2048-5X10NMOL
	2048-10NMOL
Appearance:	Orange Powder/Solid
Revision:	1.1 (June 2022)

Congratulations on your acquisition of a revolutionary new gold labeling reagent: Mono-DBCO (dibenzocyclooctyne)-Undecagold. With this biorthogonal reagent, you can label your azide or any azide-containing molecule of interest, including peptides, proteins, oligonucleotides, and cellular components, with Undecagold gold nanoparticles for high-resolution electron microscope localization and detection. Undecagold is the smallest gold label 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 a specially developed gold label, prepared using a discrete gold compound rather than a colloid.[1] This kit contains the Undecagold particle with a DBCO (**D**i**B**enzo**C**yclo**O**ctyne) 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 a 1,3-dipolar cycloaddition reaction known as a strain-promoted alkyne azide cycloaddition (SPAAC), which does not require a copper (I) catalyst[2,3] (Figure 1). The advantages of the copper-free azide-alkyne cycloaddition (SPAAC) reaction are (1) that it is biorthogonal: azides and alkynes react selectively only with each other, and not with any naturally occurring cellular components; and (2) the use of strain-promoted alkyne azide cycloaddition (SPAAC) avoids the cytotoxicity of copper (I) catalyzed cycloadditions, making this reagent fully compatible with labeling components of vital processes in living cells and tissues.

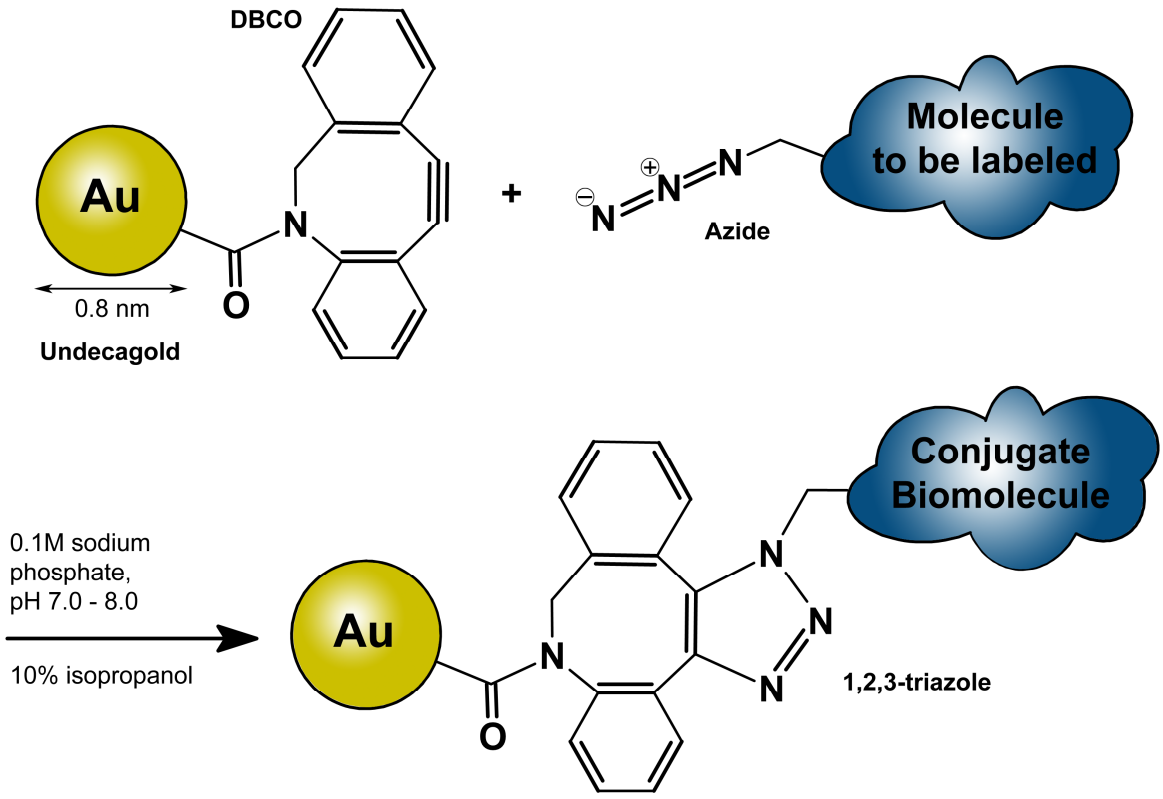

Figure 1: Schematic showing mono-DBCO-Undecagold labeling of an azide via 1,3-dipolar strain-promoted alkyne azide cycloaddition (SPAAC).

Mono-Alkyne-Undecagold reagent as supplied has been lyophilized from 0.1 M sodium phosphate at pH 8.0. The solid labeling reagent should be stored at -20°C. Dissolution in 0.5 mL (50 nmol size) or 0.1 mL (10 nmol size) of deionized water will produce a solution of activated Undecagold in 0.1 M sodium phosphate at pH 8.0 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. Even a small amount of residual thiol reagent can severely limit the performance of Undecagold.

OTHER CAUTIONS

Although Undecagold is usually stable under demanding conditions, including pH values lower than 4 or ionic strengths above 1 M, Undecagold reagents, conjugates or labeled specimens 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 should be avoided, and the use of low temperature embedding media (e.g. Lowicryl) is recommended if labeling before embedding.[4] It is not recommended to bake tissue blocks with Undecagold. If your experiment requires higher temperature embedding, then silver or gold enhancement should be performed before embedding.

CLICK REACTION CONDITIONS

EXAMPLE PROTOCOL

This section contains a general protocol for strain-promoted alkyne azide cycloaddition (SPAAC) click reactions.[3,5] DBCO-azide (SPAAC) conjugation reactions generally proceed quickly with high selectivity at pH values between 7.0 and 8.0, close to physiological pH, and we recommend dissolving the molecule to be labeled in 0.1 M sodium phosphate buffer at pH 7 in order to produce a pH close to physiological pH (7.4) when the solutions are mixed.

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

Procedure using 50 nmol size (catalog number 2048-50NMOL):

1. Prepare a 500 µM solution of the azide-modified molecule to be labeled in 0.1 M phosphate pH 7.0 containing 10% DMSO.

   If you are labeling a protein or other biomolecule and plan to separate the Undecagold-labeled product by gel filtration liquid chromatography, prepare the following volumes of protein solution:

   (a) If the azide-modified protein to be labeled is larger than 8 kDa (8,000 MW), prepare 20 µL (0.02 mL), or 10 nmol (to give a 5-fold excess of Mono-DBCO-Undecagold).

   (b) If the azide-modified protein is 3 – 8 kDa (3,000 – 8,000 MW), prepare 60 µL (0.06 mL), or 30 nmol (to give a 1.67-fold excess of Mono-DBCO-Undecagold)

   (c) If the azide-modified protein is smaller than 3 kDa (3,000 MW), prepare 300 µL (0.3 mL), or 150 nmol (to give a 3-fold excess of azide-modified protein).

   These ratios are intended to ensure that when the reaction mixture is separated, the larger of the two reagents is the limiting reagent, and the smaller of the two reagents, which is more easily separated from the larger conjugate, is present in excess.

   If you are labeling an oligonucleotide and plan to separate the Undecagold-labeled product by ethanol precipitation, prepare 100 µL, or 50 nmol (to give a 1 : 1 ratio of Mono-DBCO-Undecagold : azide-modified oligonucleotide). This should ensure the highest yield of Undecagold conjugate and the smallest amounts of unreacted starting materials.

2. Add 0.5 mL deionized water to one vial of 50 nmol Mono-DBCO-Undecagold. Vortex. It will yield 0.5 mL of 100 µM Mono-DBCO-Undecagold in 0.1 M phosphate pH 8.0. If reagent does not fully dissolve, add 0.05 mL of isopropanol and vortex again.

3. Add the 500 µM solution of the azide-modified molecule to be labeled, prepared in step 1, to the 0.5 mL of Mono-DBCO-Undecagold solution. Vortex the mixture to ensure thorough solution and mixing.

4. Incubate the reaction mixture, either

   (a) on a shaker at room temperature for 1 hour, or

   (b) at 2 – 8°C overnight in the refrigerator

5. Purify Undecagold-conjugated peptide or proteins from unlabeled peptide or protein or excess Undecagold reagents using gel filtration liquid chromatography. Use a gel with an appropriate molecular weight separation range for your experiment, e.g.

Superose-12 or Superdex-75 for larger proteins, Biorad P-30, or Superdex-PG30 for smaller molecules. For oligonucleotide conjugates, perform ethanol precipitation. The resulting conjugated proteins in lysate are ready for downstream processing or analysis.

Procedure using 10 nmol size (catalog number 2048-5X10NMOL or 2028-10NMOL):

1. Prepare a 500 µM solution of the azide-modified molecule to be labeled in 0.1 M phosphate pH 7.0 containing 10% DMSO.

   If you are labeling a protein or other biomolecule and plan to separate the Undecagold-labeled product by gel filtration liquid chromatography, prepare the following volumes of protein solution:

   (a) If the azide-modified protein to be labeled is larger than 8 kDa (8,000 MW), prepare 4 µL (0.004 mL), or 2 nmol (to give a 5-fold excess of Mono-DBCO-Undecagold).

   (b) If the azide-modified protein is 3 – 8 kDa (3,000 – 8,000 MW), prepare 12 µL (0.012 mL), or 6 nmol (to give a 1.67-fold excess of Mono-DBCO-Undecagold)

   (c) If the azide-modified protein is smaller than 3 kDa (3,000 MW), prepare 60 µL (0.06 mL), or 20 nmol (to give a 3-fold excess of azide-modified protein).

   These ratios are intended to ensure that when the reaction mixture is separated, the larger of the two reagents is the limiting reagent, and the smaller of the two reagents, which is more easily separated from the larger conjugate, is present in excess.

   If you are labeling an oligonucleotide and plan to separate the Undecagold-labeled product by ethanol precipitation, prepare 100 µL, or 50 nmol (to give a 1 : 1 ratio of Mono-DBCO-Undecagold : azide-modified oligonucleotide). This should ensure the highest yield of Undecagold conjugate and the smallest amounts of unreacted starting materials.

2. Add 0.1 mL deionized water to one vial of 10 nmol Mono-DBCO-Undecagold. Vortex. It will yield 0.1 mL of 100 µM Mono-DBCO-Undecagold in 0.1 M phosphate, pH 8.0. If reagent does not fully dissolve, add 0.01 mL of isopropanol and vortex again.

3. Add the 500 µM solution of the azide-modified molecule to be labeled, prepared in step 1, to the 0.1 mL of Mono-DBCO-Undecagold solution. Vortex the mixture to ensure thorough solution and mixing.

4. Incubate the reaction mixture, either

   (a) on a shaker at room temperature for 1 hour, or

   (b) at 2 – 8°C overnight in the refrigerator

5. Purify Undecagold conjugated peptide or proteins from unlabeled peptide or protein or excess Undecagold reagents using gel filtration liquid chromatography using a gel with an appropriate molecular weight separation range for your experiment, e.g. Superose-12 or Superdex-75 for larger proteins, Biorad P-30, or Superdex-PG30 for smaller molecules. For oligonucleotide conjugates, perform ethanol precipitation. The resulting conjugated proteins in lysate are ready for downstream processing or analysis.

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

CHARACTERIZATION OF UNDECAGOLD CONJUGATES

The purified Undecagold conjugates of peptides, or 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 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 Undecagold using the molar extinction coefficient of Undecagold (47,000 $M^{-1}cm^{-1}$ 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 Undecagold, which may be calculated using the values for $\gamma_{gold\ (\ 280\ nm/420nm)}$ or $\gamma_{gold\ (260\ nm/420nm)}$ provided in the product specification sheet multiplied by A_{420nm}.

[Undecagold] = $[A_{420nm}]/47{,}000$

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

or

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

DOL = [Undecagold]/[Protein]

or

DOL = [Undecagold]/[oligonucleotide]

Detailed instructions for this calculation, as well as an example of the Undecagold UV/visible spectrum, are available on our web site at https://www.nanoprobes.com/guides/LGuide4.html.

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 5,000 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 or until colored band(s) develop. 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 FOR USING MONO-DBCO-UNDECAGOLD REAGENTS

- Undecagold is an extremely uniform 0.8 nm diameter gold particle (±10%).

- Mono-DBCO-Undecagold reacts with an azide to form a 1,2,3-triazole conjugate via SPAAC.

- 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 DIRECT VIEWING OF 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 AND GOLD 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/references/index.html.