PALMITOYL UNDECAGOLD*

Nanoprobes

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PRODUCT INFORMATION

PALMITOYL UNDECAGOLD

Product Name:	PALMITOYL UNDECAGOLD
Catalog Number:	4022
Appearance:	orange-yellow solid
Revision:	1.1 (March 2000)

GENERAL INFORMATION

Palmitoyl-UNDECAGOLD consists of the 0.8 nm UNDECAGOLD particle[1] covalently linked to a single palmitoyl molecule. Conjugation is via an amide linkage to the carboxylic group at the head of the molecule. It is intended as a lipid label for use with micelles and other dual-phase systems. Its structure is shown in figure 1:

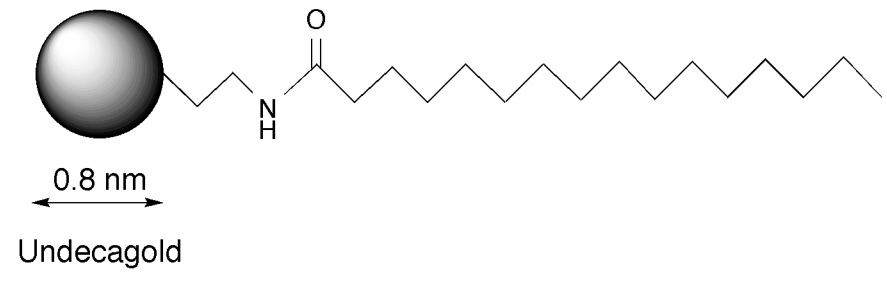

Figure 1: Structure of Palmitoyl UNDECAGOLD (not shown to scale).

Palmitoyl UNDECAGOLD is supplied as a solid, dried from dichloromethane solution. It should be frozen upon receipt, and stored at -20°C. The extinction coefficient at 420 nm is shown below:

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.

* US Patent pending.

INSTRUCTIONS FOR USE

Palmitoyl UNDECAGOLD is hydrophobic, and can insert into organic phases in systems such as micelles.[2] It is soluble in methanol and in methanol-trichloromethane and methanol-dichloromethane mixtures. Once incorporated into micelles or other structures, it may be used according to the procedures required by individual experiments in the same manner as unlabeled palmitic acid or its derivatives.

WAVELENGTH (nm)	EXTINCTION COEFFICIENT*
420	0.471×10^5

*Measured for 5×10^{-6} M solution in methanol.

GENERAL CONSIDERATIONS FOR STAINING WITH UNDECAGOLD REAGENTS

For microscopy, colloidal gold methodologies may be used successfully with UNDECAGOLD labeled reagents. Similar dilutions and blocking agents are appropriate.

The major difference will be in the results:

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

UNDECAGOLD conjugates are the smallest gold probes commercially available and will penetrate and reach antigens inaccessible to other gold probes.

UNDECAGOLD conjugates are chromatographically purified through gel filtration columns. There are absolutely no aggregates or other molecular weight impurities. This is in sharp contrast to other colloidal gold conjugates that usually are prepared by centrifugation to remove the largest aggregates and frequently contain smaller aggregates.

Close to 1 UNDECAGOLD particle to 1 lipid molecule make this product distinct from the 0.2 - 10 variable stoichiometry of colloidal gold conjugates.

UNDECAGOLD particles do not have affinity to proteins as do other other colloidal golds. This reduces background and false labeling.

USING STAINS WITH UNDECAGOLD

Because the 0.8 nm UNDECAGOLD particles are so small, over staining with OsO_4, uranyl acetate or lead citrate may tend to obscure direct visualization of individual UNDECAGOLD particles. Three recommendations for improved visibility of UNDECAGOLD are:

1. Use of reduced amounts or concentrations of usual stains.
2. Use of lower atomic number stains such as NANOVAN™, a Vanadium based stain.
3. Enhancement of UNDECAGOLD with silver developers, such as LI SILVER or HQ SILVER.

THIOL CAUTION

UNDECAGOLD particles degrade upon exposure to concentrated thiols such as ß-mercaptoethanol or dithiothreitol. If such reagents must be used, concentrations should be kept below 1 mM and exposure restricted to 10 minutes or less.

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 Eelectron Microscope (STEM) is required for visualization; in a conventional TEM the UNDECAGOLD particles are not visible. With careful work, however, 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 UNDERCAGOLD in the STEM has been described in the literature.[2] Image at approximately 200 e Å^{-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 deposition resulting in a dense particle 2-20 nm in size or larger depending on development time. However, silver enhancement will be slower and much less uniform than with larger gold particles such as NANOGOLD™. If specimens are to be embedded, silver 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 deposition in the same manner as gold and produce non-specific staining.

Our LI SILVER silver enhancement system is convenient and not light sensitive, 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.

Specimens must be thoroughly rinsed with deionized water before silver 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. These are often light-sensitive and will give non-specific staining. To prepare the developer, mix equal amounts of the enhancer and initiator immediately before use. UNDECAGOLD will nucleate silver deposition resulting in a dense particle 2-20 nm in size or larger depending on development time. Use nickel grids (not copper).

Silver enhancement is performed as follows:

1. Rinse with deionized water (2 X 5 mins).
2. Float grid with specimen on freshly mixed developer for 1-4 minutes, or as directed in the instructions for the silver reagent. More or less time can be used to control particle size. A series of different development times should be tried, to find the optimum time for your experiment.
3. Rinse with deionized water (3 X 1 min).
4. Mount as usual.

STAINING AND SILVER ENHANCEMENT WITH UNDECAGOLD FOR LIGHT MICROSCOPY

Features labeled with UNDECAGOLD will be stained black in the light microscope upon silver enhancement. Different development times should be tried to determine which is best for your experiment. The procedure for immunolabeling is similar to that for EM; a suitable procedure is given below.

Samples must be rinsed with deionized water before silver enhancement. This is because the reagent contains silver ions in solution, which react to form a precipitate with chloride, phosphate and other anions which are components of buffer solutions. The procedure for immunolabeling with UNDECAGOLD and silver enhancement is given below.

1. Rinse with deionized water (3 X 1 min).
2. Develop specimen with freshly mixed developer for 5-20 minutes, or as directed in the instructions for the silver reagent. More or less time can be used to control intensity of signal. A series of different development times may be used, to find the optimum enhancement for your experiment; generally a shorter antibody incubation time will require a longer silver development time.
3. Rinse with deionized water (2 X 5 mins).
4. The specimen may now be stained if desired before examination, with usual reagents.

To obtain an especially dark silver signal, the silver enhancement may be repeated with a freshly mixed portion of developer.

REFERENCES

1. Hainfeld, J. F., in "Colloidal Gold: Principles, Methods and Applications;" M. A. Hayat, ed.; Academic Press, San Diego, 1989; pp 413-435.

2. Lipka, J. J., Hainfeld, J. F., and Wall, J. S., *J. Ultrastruct. Res.*, 84, 120 (1983).

Technical Assistance Available.

For a complete list of references citing our products, please visit our world-wide-web site at http://www.nanoprobes.com/Ref.html.