DPPE-NANOGOLD®*

Nanoprobes

95 Horse Block Road, Yaphank NY 11980-9710
Tel: (877) 447-6266 (Toll-Free in US) or (631) 205-9490 Fax: (631) 205-9493
Tech Support: (631) 205-9492 tech@nanoprobes.com
www.nanoprobes.com

PRODUCT INFORMATION

DIPALMITOYLPHOSPHATIDYL ETHANOLAMINE-NANOGOLD®

Product Name:	DIPALMITOYLPHOSPHATIDYL ETHANOLAMINE-NANOGOLD®
Catalog Number:	4021
Appearance:	Dark brown solid
Revision:	1.2 (March 2000)

GENERAL INFORMATION

DPPE-NANOGOLD® consists of the 1.4 nm NANOGOLD® particle covalently linked to a single molecule of dipalmitoyl phosphatidyl ethanolamine. Conjugation is via the amine group on the ethanolamine head of the molecule. It is intended as a lipid label for use with micelles and other dual-phase systems[1,2] in the same manner as the previously described undecatungstate membrane label[3]. It has been used to prepare gold-decorated liposomes[1], and also to track the dellivery of a liposomally encapsulated antifungal drug[2]. Its structure is shwon in figure 1:

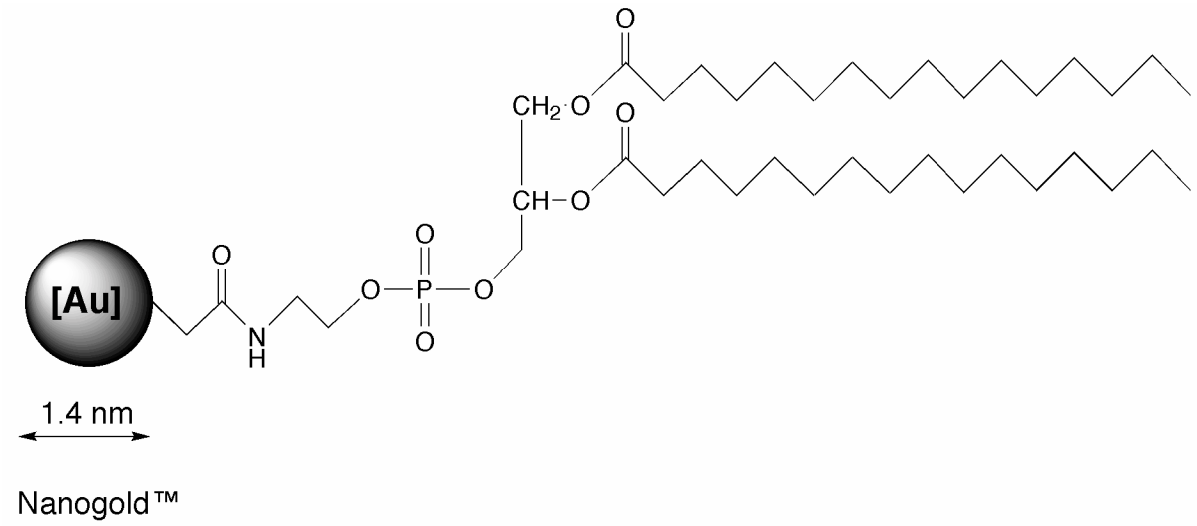

Figure 1: Structure of dipalmitoyl phosphatidyl ethanolamine-NANOGOLD® (not shown to scale).

DPPE-NANOGOLD® is supplied as a solid, dried from methanol solution. It should be refrigerated upon receipt, and stored at 2 - 8°C.

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

DPPE-NANOGOLD® is hydrophobic, and can insert into organic phases in systems such as micelles.[1] It is soluble in methanol and in methanol-trichlrormethane and methanol-dichloromethane mixtures. Extinction coefficients at specific wavelengths are given below for methanol solution:

WAVELENGTH (nm)	EXTINCTION COEFFICIENT*
280	2.25×10^5
420	1.12×10^5

*Measured for 5 X 10-6 M solution in methanol.

Heavily gold-labeled liposomes may be formed by dissolving the Nanogold®-DPPE in chloroform, mixing with water, evaporating the chloroform, then using sonication or other usual preparative methods to form vesicles[1]. Nanogold®-DPPE may also be diluted with unlabeled DPPE or with other lipids and used to prepare less heavily labeled liposomes. Incorporation of 0.1 to 1 % Nanogold®-DPPE with an unlabeled lipid is usually appropriate for preparing Nanogold®-labeled liposomes with the same properties and morphology as those prepared without the gold label[2]. 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 DPPE.

GENERAL CONSIDERATIONS FOR STAINING WITH NANOGOLD® REAGENTS

For microscopy, colloidal gold methodologies may be used successfully with NANOGOLD® immunoreagents. Similar dilutions and blocking agents are appropriate.

The major difference will be in the results:

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

NANOGOLD® conjugates are the smallest gold probes commercially available which are visible in a standard transmission electron microscope (TEM), and will penetrate and reach antigens inaccessible to other gold probes.

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

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

NANOGOLD® develops better with silver than do most other colloidal golds giving it higher sensitivity. Silver enhancement can be used to make the immunolabeling useful for electron microscopy, light microscopy, and immunoblotting with improved results.

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 NANOVAN™, a Vanadium based stain.

3. Enhancement of NANOGOLD® with silver developers, such as LI SILVER or HQ SILVER.

TEMPERATURE CAUTION

NANOGOLD® particles are not stable above 50°C. Best results are obtained at room temperature or 4°C. Avoid 37°C incubations. Use low temperature embedding media (e.g., Lowicryl) if labeling before embedding; do not bake tissue blocks with NANOGOLD®. If your experiment requires higher temperature embedding, then silver enhancement should be performed before embedding.

THIOL CAUTION

NANOGOLD® 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 DIRECT VIEWING OF NANOGOLD® IN THE ELECTRON MICROSCOPE

For most work, silver 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 sampl;es 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 must 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 X 10^6 with binoculars), over focus, under focus, then set the objective lens to in focus. 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 enhancement to give particles easily seen at lower magnification.

SILVER ENHANCEMENT OF NANOGOLD® FOR EM

NANOGOLD® will nucleate silver deposition resulting in a dense particle 2-80 nm in size or larger depending on development time. 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. With NANOGOLD® reagents, low-temperature resins (eg Lowicryl) should be used and the specimens kept at or below room temperature until after silver development has been completed. Silver development is recommended for applications of NANOGOLD® in which these stains are to be used, otherwise the NANOGOLD® particles may be difficult to visualize against the stain.

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. NANOGOLD® 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).

The relevent procedure for immunolabeling should be followed up to step 7 as described above. Silver enhancement is then performed as follows:

1. Rinse with deionized water (2 X 5 mins).
2. OPTIONAL (may reduce background): Rinse with 0.02 M sodium citrate buffer, pH 7.0 (3 X 5 mins).
3. Float grid with specimen on freshly mixed developer for 1-8 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. With HQ silver, a development time of 6 min. gives 15-40 nm round particles.
4. Rinse with deionized water (3 X 1 min).
5. Mount and stain as usual.

Fixing with osmium tetroxide may cause some loss of silver; if this is found to be a problem, slightly longer development times may be appropriate.

STAINING AND SILVER ENHANCEMENT WITH NANOGOLD® FOR LIGHT MICROSCOPY

Features labeled with NANOGOLD® 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 NANOGOLD® and silver enhancement is given below.

1. Rinse with deionized water (3 X 1 min).
2. OPTIONAL (may reduce background): Rinse with 0.02 M sodium citrate buffer, pH 7.0 (3 X 5 mins).
3. 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.
4. Rinse with deionized water (2 X 5 mins).
5. 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.

IMMUNOBLOTTING

The basic procedure for gold immunoblotting has been described by Moeremans et al;[4] this may be used for experiments in which NANOGOLD® reagents are used in biological systems as part of blotting techniques. For best results, the membrane should be hydrated before use by simmering in gently boiling water for 15 minutes. Best results are obtained when the antigen is applied using a 1 µl capillary tube. The procedure for immunoblots is as follows:

1. Spot 1 µl dilutions of the target in buffer 4 onto hydrated nitrocellulose membrane. Use an antigen concentration range from 100 to 0.01 pg / µl.
2. Block with buffer 1 for 30 minutes at 37°C.
3. Incubate with a solution of the DPPE-NANOGOLD® product (such as a micelle) in buffer 2 for 2 hours at room temperature.
4. Rinse with buffer 3 (3 X 5 mins), then buffer 4 (2 X 5 mins).
5. OPTIONAL (may improve sensitivity): Postfix with glutaraldehyde, 1 % in buffer 4 (10 mins).
6. Rinse with deionized water (2 X 5 mins).
7. OPTIONAL (may reduce background): Rinse with 0.05 M EDTA at pH 4.5 (5 mins).
8. Develop with freshly mixed silver developer for 20-25 minutes or as directed in the instructions for the silver reagent, twice. Rinse thoroughly with deionized water between developments to remove all the reagent.
9. Rinse several times with deionized water.

CAUTION: NANOGOLD® 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.

Buffer 1:
20 mM phosphate
150 mM NaCl
pH 7.4
4% BSA (bovine serum albumin)
2 mM sodium azide (NaN_3)

Buffer 2:
20 mM phosphate
150 mM NaCl
pH 7.4
0.8% BSA
1% normal serum (normal goat serum, or other serum not targeted by antibodies used in earlier steps)
0.1% gelatin (Type B, approx. 60 bloom)
Optional, may reduce background:
0.5 M NaCl
0.05% Tween 20

Buffer 3: 20 mM phosphate
150 mM NaCl
pH 7.4
0.8% BSA (bovine serum albumin)
2 mM sodium azide (NaN_3)

Buffer 4 (PBS):
20 mM phosphate
150 mM NaCl
pH 7.4

Other procedures may be used; for example the NANOGOLD® reagent may be used as a tertiary labeled probe. If antibody incubation steps are used, rinse with buffer 3 (3 X 10 mins) after incubation.

REFERENCES

1. Hainfeld, J. F.: In *Proc 54th Ann. Mtg. Micros. Soc. Amer.*, G. W. Bailey, J. M. Corbett, R. V. W. Dimlich, J. R. Michael and N. J., Zaluzec (Eds.). San Francisco Press, San Francisco, CA, pp. 898-899 (1996).

2. Adler-Moore, J.: *Bone Marrow Transplantation*, 14, S3-S7 (1994).

3. Hainfeld, J. F.; Lipka, J. J., and Quaite, F. E.; *J. Histochem. Cytochem.*, 38, 1795 (1990).

4. Moeremans, M. et al., *J. Immunol. Meth*. 74, 353 (1984).

Technical Assistance Available.

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