NANOGOLD®

Nanoprobes

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PRODUCT INFORMATION SHEET

POSITIVELY CHARGED NANOGOLD®

Product Name:	POSITIVELY CHARGED NANOGOLD®
Catalog Number:	2022
Appearance:	Brown solid
Revision:	1.2 (March 2000)

GENERAL INFORMATION

NANOGOLD® (NG) is a revolutionary new type of gold particle, prepared using a process that gives precise control over its size distribution and surface properties. NANOGOLD® particles are a uniform 1.4 nm in diameter, making them a suitable calibration standard for electron microscopy. They do not aggregate, as do colloidal gold products, nor do they possess affinity for proteins as colloidal gold particles do.[1] Each molecule of POSITIVELY CHARGED NANOGOLD® bears several primary amine groups; these may ionize and assume a positive charge, and these particles may therefore be used to label specific regions of cells or biomolecules with a net negative charge. POSITIVELY CHARGED NANOGOLD® has been used as a marker to study the yest endocytic pathway.[1] They may also be used for the attachment of labels, via usual cross-linking protocols.

POSITIVELY CHARGED NANOGOLD® particles should be frozen upon receipt, and stored at -20°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.

PRODUCT SPECIFICATIONS

POSITIVELY CHARGED NANOGOLD® is supplied as a solid, lyophilized from methanol: the 1 ml vial contains 30 nmol. It is purified by gel filtration, and is stable under a wide range of pH confitions. It is soluble in water and aqueous buffers, and also in organic solvents such as alcohols, acetone, dichloromethane and similar solvents. Solubility in non-polar organic solvents may be improved by treatment with a small amount of base. Each molecule of POSITIVELY CHARGED NANOGOLD® contains approximately 6 primary amine groups.

Extinction coefficients at specific wavelengths are given below for methanol solution:

WAVELENGTH (nm)	EXTINCTION COEFFICIENT*
280	2.24×10^5
420	1.12×10^5

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

THIOL CAUTION

NANOGOLD® particles degrade upon exposure to thiols such as ß-mercaptoethanol or dithiothreitol. A small amount of residual thiol reagent can severely limit the performance of NANOGOLD®. Thiol compounds used in the reduction of protein molecules (or other biomolecules) should be separated from the protein by gel filtration chromatography before NANOGOLD® conjugation. Use a gel, such as Amicon GH-25, which has an exclusion cut-off at molecular weight 3,000. Dialysis does NOT provide acceptable purification in this application.

TEMPERATURE CAUTION

Although NANOGOLD® is usually stable,[3] under some conditions labeled specimens or conjugates may not be 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;[4] do not bake tissue blocks with NANOGOLD®. If your experiment requires higher temperature embedding, then silver enhancement should be performed before embedding.

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.[5]
3. Enhancement of NANOGOLD® with silver developers, such as LI SILVER or HQ SILVER.

INSTRUCTIONS FOR USE

The product is supplied as a lyophilized pure solid; no buffers or other salts or residues are present. The brown solid may be dissolved directly into the solvent or buffer to be used.

This product may be used to label cellular features or biomolecules with a predominantly negative charge. The amine groups may also be cross-linked to other molecules using commercially available hmobifunctional or heterobifunctional cross-linkers.

SPECIAL CONSIDERATIONS FOR VIEWING NANOGOLD® IN THE ELECTRON MICROSCOPE

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. 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® PARTICLES

NANOGOLD® will nucleate silver deposition resulting in a dense particle 2-20 nm in size or larger depending on development time. 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.[6]

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).

Silver enhancement is 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 1-4 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.
4. Rinse with deionized water (3 X 1 min).

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.

NOTE: Treatment with osmium tetroxide followed by uranyl acetate staining can lead to much more drastic loss of the silver enhanced NANOGOLD® particles. This may be prevented by gold toning:[7]

1. After silver enhancement, wash thoroughly with dionized water.

2. 0.05 % gold chloride: 10 minutes at 4°C.
3. Wash with deionized water.
4. 0.5 % oxalic acid: 2 mins at room temperature.
5. 1 % sodium thiosulfate (freshly made) for 1 hour.
6. Wash thoroughly with deionized water and embed according to usual procedure.

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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.