

GOLDENHANCE™ - EM



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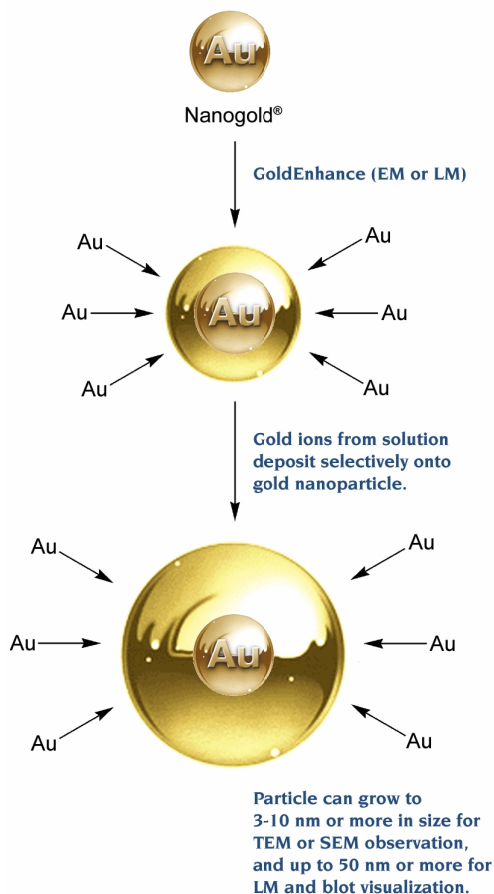
INSTRUCTIONS

GOLDENHANCE™ - EM Formulation

Product: GoldEnhance™ - EM Formulation
Catalog Number: 2113
Appearance: Colorless solution
Revision: 1.5 (January 2009)

INTRODUCTION

This novel, high-quality autometallographic enhancement reagent may be used in the same manner as conventional silver enhancement reagents. However, instead of depositing silver, this product selectively deposits gold onto Nanogold® particles or colloidal gold particles.¹⁻³ This has a number of advantages for electron microscopy, light microscopy and membrane blotting experiments:



Why gold?

Gold has several important advantages for electron microscopy:

- Higher density; better contrast than silver.
- Improved backscatter detection of enlarged particles - more useful for SEM.
- Lower background than silver enhancement in some cases. Autonucliation is minimal even after 1-2 hours.
- High resolution.
- Gold is not etched by osmium tetroxide: gold toning or other treatment is not necessary.
- Low viscosity for easy and accurate mixing of components.
- Milder pH conditions than silver enhancement: Goldenhance is used at near neutral pH.
- Goldenhance can be used in physiological buffers - gold is not precipitated by halides as silver is (however, rinsing with water first is still recommended).
- Excellent shelf life.

Figure 1: Enlargement of Nanogold® with GoldEnhance™.

PRODUCT INFORMATION

This reagent consists of 2 ml Solution A (enhancer), 2 ml Solution B (activator), 2 ml Solution C (initiator), and 2 mL Solution D (buffer), sufficient for up to 200 grids (using 40 μ L per grid). The reagent is formed by combining equal volumes of Solution A and Solution B, then Solution C followed by Solution D. The mixture should be prepared immediately before use. For optimum results, we recommend waiting 5-10 minutes after mixing A and B before adding C and D, although the reagent will produce successful enhancement if C and D are added immediately or up to two hours later. Nanogold[®] or colloidal gold nucleates deposition of gold to give electron-dense enlarged colloidal particles in the electron microscope.

Please Note: This formulation is intended principally for electron microscopy, although it may be applicable to some light microscopy or blotting applications. The alternative LM/Blot formulation (catalog number 2112) is optimized for use in light microscopy and in membrane blots.

This time period for optimum gold enhancement varies with application, but 3 to 20 minutes has been found to be optimal for enlarging the 1.4 nm Nanogold[®] particles to 3-20 nm or larger in size: 3-10 minutes will give particles from 3 to 20 nm in size, and longer development times will give larger particles up to 50 nm in size.

Store the component solutions in the refrigerator at 2-8°C. Avoid cross-contamination of the solutions: to prevent replacing the caps on the wrong bottles, the cap of the Solution A (enhancer) is green and that of Solution B (activator) is yellow, while that of Solution C (initiator) is purple and that of Solution D (buffer) is white. Avoid skin contact.

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.

Note: All components should be equilibrated to room temperature prior to the enhancement procedure.

GOLD ENHANCEMENT FOR ELECTRON MICROSCOPY

GoldEnhance[™] is prepared immediately before use by mixing equal amounts of Solution A (enhancer) and Solution B (activator), followed by a third equal volume of the Solution C (initiator), and a fourth equal volume of Solution D (buffer). For optimum results, we recommend waiting 5-10 minutes after mixing A and B before adding C, although the reagent will produce successful enhancement if C and D are added immediately or up to two hours later. The reagents are supplied in dropping bottles for easier dispensing of small amounts.

If aldehyde-containing reagents have been used for fixation, it is recommended that these be quenched before labeling. This may be achieved by incubating the specimens for 5 minutes in 50 mM glycine solution in PBS (pH 7.4). Ammonium chloride (50 mM) or sodium borohydride (0.5 - 1 mg/ml) in PBS may be used instead of glycine.

The following procedure has been found to be effective for enhancement of tissue sections for enlargement of the 1.4 nm Nanogold[®] to 3-20 nm or larger for electron microscope observation; 3-10 minutes will give particles from 3 to 20 nm in size, and longer development times will give larger particles up to 50 nm in size. However, this reagent is intended for use in a range of conditions, and different washes and development times may give better results in your system. You should follow your normal procedure up to the application of the gold conjugate; the protocol below describes the steps after this:

1. Incubate with the immunogold or Nanogold[®] conjugate according to your usual or recommended protocol.
2. Optional: postfix with 1 % glutaraldehyde in PBS.
3. Wash 3 X 5 mins with PBS with 50 mM glycine (after glutaraldehyde postfix only - to remove aldehydes)
4. Wash 3 X 5 mins in PBS-BSA.
5. Wash 3 X 5 mins in distilled water.
6. Gold enhance. Use equal amounts of the four components (Solutions A,B,C, and D); prepare about 40 μ L of reagent per grid. A convenient method is to use one drop (~10 μ L) from each bottle. After mixing, a drop may be placed on a sheet of parafilm and a grid floated on it for the required time.
 - a. First mix Solution A (enhancer: green cap) and Solution B (activator: yellow cap)
 - b. Wait 5 min.
 - c. Add Solution C (initiator: purple cap), then Solution D (buffer: white cap) and mix.
 - d. Develop for the optimal particle size (usually between 3-20 min).

- Rinse with distilled water.

PBS-BSA Buffer:

20 mM phosphate
150 mM NaCl
pH 7.4
1 % bovine serum albumin (BSA)

optional, may reduce background:

0.5 M NaCl
0.05% Tween 20

PBS Buffer:

20 mM phosphate
150 mM NaCl
pH 7.4

Notes:

- Development starts with addition of Solution C (initiator), so apply to sample as soon as possible after adding C & D to minimize autonucleation background.
- Secondary GEEM solution: for slower development, substitute the following buffer for Solution D (buffer):
0.05 M sodium phosphate
0.1 M NaCl
pH 6.1
- To obtain an especially dark signal, or for further development, develop longer or gold enhancement may be revitalized with a freshly mixed portion of GoldEnhance™ (rinse with distilled water between applications of GoldEnhance™).
- The development is not highly light sensitive, so it may be conducted under normal room lighting.

GOLD ENHANCEMENT FOR LIGHT MICROSCOPY

Note: we do not recommend the use of this reagent for light microscopy. Usually, stronger signals and higher signal-to-noise ratios may be obtained using the LM/Blot formulation of this reagent (catalog number 2112).⁴

GOLD ENHANCEMENT FOR IMMUNOBLOTS AND MEMBRANE BLOTS

Note: We do not recommend using this reagent for immunoblots or membrane blots, since stronger signals and better signal-to-noise ratios are usually obtained using the EM/Blot formulation of this reagent (catalog number 2112).⁵

REFERENCES

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Technical Assistance Available.

For a complete list of references citing this product, please visit our world-wide-web site at:

www.nanoprobes.com/Ref.html