GOLDENHANCE™ EM

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INSTRUCTIONS

GOLDENHANCE™ EM

Product:	GoldEnhance™ EM
Catalog Number:	2113
Appearance:	Colorless or yellow solution
Revision:	1.5 (October 2013)

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. 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. Autonucleation is minimal.
- 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).
- Use under normal room lighting.
- Excellent shelf life.

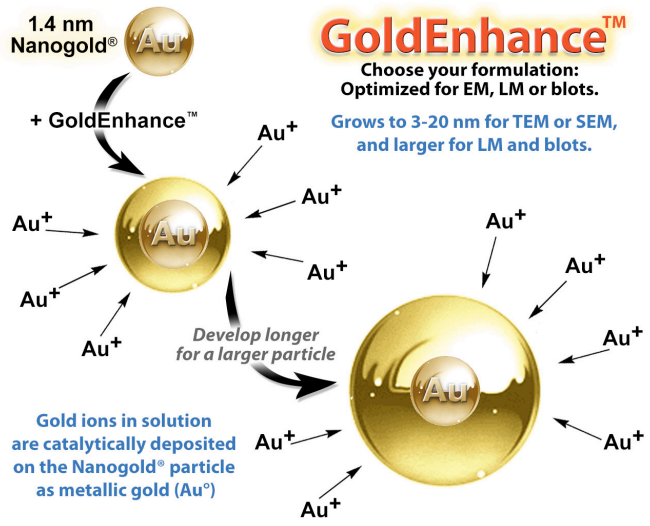

Figure 1: Enlargement of Nanogold® with GoldEnhance™ .

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 μL 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 (catalog number 2113) is intended for a fast enhancement for electron microscopy, while GoldEnhance™ EM plus (catalog number 2114) provides a slow development for better control of particle sizes for electron microscopy. The GoldEnhance™ LM (catalog number 2112) and GoldEnhance™ Blots (catalog number 2115) are optimized for use in light microscopy and in membrane blots, respectively.

This time period for optimum gold enhancement varies with application, and needs to be determined. With this formulation, 1 to 5 minutes has been found to be optimal for enlarging the 1.4 nm Nanogold® particles to 3-20 nm, 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™ EM 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 minutes after mixing A and B before adding C and D. 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; 1-5 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. Optional: 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 μL of reagent per grid. A convenient method is to use one drop (~10 μL) from each bottle. After mixing, the mixture 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 1-5 min).

7. 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
pH 7.4

Notes:

- Development starts with addition of Solution C (initiator) and Solution D (buffer), so apply to sample as soon as possible after adding C & D to minimize autonucleation background.
- Secondary GEEM solution: for slower development, use GoldEnhance™ EM plus (catalog number 2114)
- 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 light sensitive, so it may be conducted under normal room lighting.

RELATED GOLDENHANCE™ PRODUCTS

#2114 GoldEnhance™ EM plus
#2112 GoldEnhance™ LM
#2115 GoldEnhance™ Blots

REFERENCES

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2. Hacker, G.W., Hauser-Kronberger, C., Zehbe, I., Su, H., Schiechl, A., Dietze, O. and Tubbs, R.: Cell Vision, **4**, 54-65 (1997); Zehbe, I., G.W. Hacker, H. Su, C. Hauser-Kronberger, J.F. Hainfeld, and R. Tubbs. 1997. *Am. J. Pathol.*, **150**, 1553-1561 (1997).

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

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