GOLDENHANCE™ LM

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INSTRUCTIONS

GOLDENHANCE™ LM

Product:	GoldEnhance™ LM
Catalog Number:	2112
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 enhancers. However, instead of depositing silver, this product selectively deposits gold onto Nanogold® or colloidal gold particles.[1]

Why gold? Gold has several important advantages for electron microscopy, light microscopy and membrane blotting:

- Very sensitive detection with low background.
- Extremely delayed autonucleation - more convenient for multiple samples, or if sample access is restricted (e.g. automated processing).
- High resolution.
- Much faster than chemiluminescence.
- Low viscosity for easy and accurate mixing of components.
- Milder pH conditions than silver enhancement: GoldEnhance™ is used at near neutral pH, and you can adjust the pH for better sample preservation.
- Can be used in physiological buffers - gold is not precipitated by halide solutions as silver is (however, rinsing with water first is still recommended).
- Permanent staining: does not fade.
- No autofluorescence or quenching.
- Observe with brightfield optics - simpler and less expensive than fluorescence.
- Excellent shelf life.

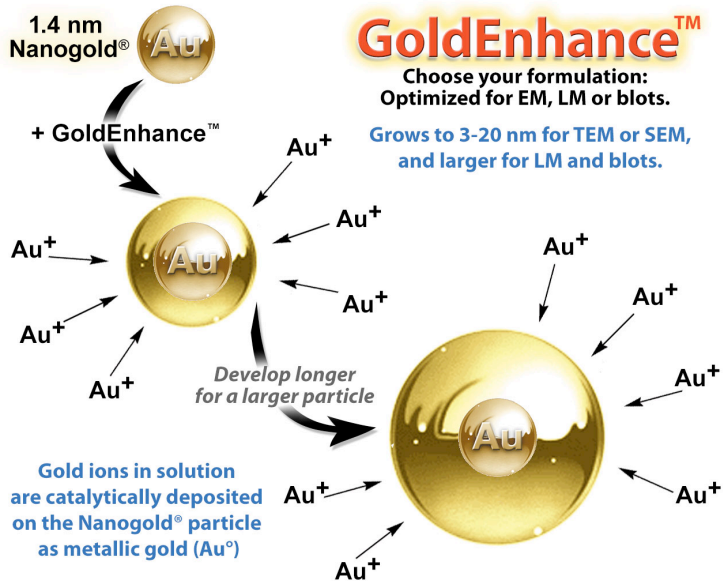

Figure 1: Enlargement of Nanogold® with GoldEnhance™ .

PRODUCT INFORMATION

This reagent consists of 15 ml Solution A (enhancer), 15 ml Solution B (activator), 15 ml Solution C (initiator) and 15 mL of Solution D (buffer), sufficient for up to 600 slides (using 100 μL per specimen). The reagent is formed by combining equal volumes of the enhancer and activator, and then adding the initiator and the buffer. 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 a dense black signal by light microscopy.

Please Note: This formulation is optimized for light microscopy. The alternative EM formulations (catalog number 2113 and 2114) are intended for EM use. GoldEnhance™ Blots (catalog number 2115) is optimized for use in membrane blots.

The time period for optimum gold enhancement varies with application, but 10 to 20 minutes has been found to be optimal for light microscopy with tissue sections.

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 the 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 LIGHT MICROSCOPY

GoldEnhance™ LM is prepared immediately before use by mixing equal amounts of Solution A (enhancer) and Solution B (activator), followed by the Solution C (initiator), and 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 receommended 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 was developed for gold enhancement of *In Situ* hybridization specimens by Hacker et al. as a modification of the Nanogold®-Silver Staining procedure.[2] It has been found to be effective for enhancement of tissue sections for light microscope observation. We have found times of 10-20 minutes give optimal results; however, this reagent is intended to function in a wide range of conditions, and different washes and development times may give better results in your application. You should follow your normal procedure up to the application of the gold conjugate; the protocol below describes the steps after this:

1. Incubate the sections with Nanogold® or colloidal gold conjugate according to current protocols or using the buffers, concentrations and protocols recommended for the conjugate.
2. Wash in PBS pH 7.6, 2 times 5 min each.
3. Wash in PBS-gelatin pH 7.6 for 5 min.
4. Repeatedly wash in distilled water for at least 10 min altogether, the last 2 rinses in ultrapure water (EM-grade).
5. Prepare GoldEnhance™ using equal amounts of the four components (Solutions A,B,C, and D); prepare about 100 μL per slide.
    a. Dispense Solution A (enhancer: green cap) into a clean tube or dish, add Solution B (activator: yellow cap), and mix thoroughly.
    b. Wait 5 minutes.
    c. Add Solution C (initiator: purple cap) and Solution D (buffer) and mix thoroughly.
    d. Apply 100 μL, or amount sufficient to cover the specimen.
    e. Develop specimen for 10 - 20 minutes. More or less time can be used to control particle size and intensity of signal.
6. When optimum staining is reached, immediately stop by rinsing carefully with deionized water.

PBS-Gelatin Buffer:

20 mM phosphate
150 mM NaCl
pH 7.6

optional, may reduce background:

0.1% gelatin (high purity)
0.5 M NaCl
0.05% Tween 20

PBS Buffer:

20 mM phosphate
150 mM NaCl
pH 7.6

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 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 can be conducted under normal room lighting, or viewing by light microscopy.
- Some users reported good development omitting the use of Solution D (buffer), but deposition times are then slower.

RELATED GOLDENHANCE™ PRODUCTS

#2114	GoldEnhance™ EM plus
#2113	GoldEnhance™ EM
#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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