

# HQ SILVER™



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## PRODUCT INFORMATION

### HQ SILVER™ ENHANCEMENT KIT

Product Name: HQ SILVER™  
Catalog Number: 2012  
Appearance: A (Initiator): Pale yellow-brown solution; B (Moderator): viscous pale brown solution; C (Activator): Viscous pale brown solution  
Revision: 2.5 (January 2009)

## GENERAL INFORMATION

HQ SILVER™ is a high-quality silver enhancement system for use with NANOGOLD® reagents, and is specially formulated for EM or light microscopy. HQ SILVER™ includes a protective colloid, which delays self-nucleation, reduces background staining and ensures uniform results with high signal-to noise ratio;<sup>1,2</sup> neutral pH assures excellent structural preservation. The system consists of 15 ml initiator, 15 ml moderator, and 15 ml activator; the reagent is formed by combining equal volumes of the moderator, initiator, and then the activator solutions. The mixture is usable only for a defined time period, as given below, and should therefore be prepared immediately before use. HQ SILVER™ is nucleated by NANOGOLD® particles, resulting in the precipitation of metallic silver and the formation of a black signal.

Silver enhancement is time-dependent: for the first time period the reaction is highly specific for gold particles. NANOGOLD® particles will nucleate the deposition of dense silver particles which will enlarge rapidly in this period. The rate of growth of these particles will decline with time as their surface area increases. The enhancement time is the time required to obtain an adequate amplification of the NANOGOLD® signal without background staining. After a certain time beyond the enhancement time, silver may be precipitated spontaneously by self-nucleation, producing background signal.

This time period varies with temperature. At 16°C the developer solution is stable (no self-nucleation occurs) for at least 20 minutes; at 20°C, the solution is stable for at least 15 minutes, and at 24°C for at least 10 minutes. After this time, background staining may be observed. For applications where a very high degree of enhancement is to be combined with low background staining, enhancement may be repeated with a fresh portion of the enhancement mixture: development will continue but self-nucleation will be very low since the self-nucleation process restarts with each freshly mixed portion of developer.

Store the component solutions at -20°C; thaw and allow to equilibrate to room temperature before use. The solutions are light-sensitive! Silver enhancement must be carried out in a darkroom or light-tight box. A safelight or other darkroom light may be used. Do not expose to heat. Avoid contact with metallic objects, since these can induce silver precipitation. Avoid cross-contamination of the enhancer and initiator solutions: to prevent replacing the caps on the wrong bottles, the cap of the initiator (solution A) is red, while that of the activator (solution C) is blue. Avoid skin contact: the silver enhancement reagents will stain skin.

**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.

## **HQ SILVER ENHANCEMENT FOR EM**

**IMPORTANT:** solutions must be thawed and allowed to thermally equilibrate to room temperature before use to ensure uniform, reproducible results. 2 hours is recommended.

The procedures used for this method have been described by Burry.<sup>1</sup> HQ SILVER™ is prepared immediately before use: equal amounts of each component (e.g. two drops of each) should be used. Mix initiator (solution A) and moderator (solution B) thoroughly, then add activator (solution C) and mix thoroughly again. The reagents are supplied in dropping bottles for easier dispensing of small amounts (for example, two drops from each bottle gives a suitable amount of developer. The moderator and activator are viscous; mixing will be easier if the components are allowed to reach room temperature first. NANOGOLD® will nucleate silver deposition resulting in a dense particle 2-10 nm in size or larger depending on development time. Silver enhancement should be performed in a darkroom or darkened box. Use nickel grids (not copper).

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.

If aldehyde-containing reagents have been used for fixation, these should be quenched before immunolabeling. 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. 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, which are often light-sensitive and will give non-specific staining. Use nickel grids (not copper).

## **Cells in Suspension**

1. Optional fixing of cells: e.g., with glutaraldehyde (0.05 - 1% for 15 minutes) in PBS. Do not use Tris buffer since this contains an amine. After fixation, centrifuge cells (e.g. 1 ml at 10<sup>7</sup> cells/ml) at 300 X g, 5 minutes; discard supernatant; resuspend in 1 ml buffer. Repeat this washing (centrifugation and resuspension) 2 times.
2. Incubate cells with 0.02 M glycine in PBS (5 mins). Centrifuge, then resuspend cells Buffer 1 (specified below).
3. Place 50 - 200 ml of cells into Eppendorf tube and add 5 - 10 ml of primary antibody (or antiserum). Incubate 30 minutes with occasional shaking (do not create bubbles which will denature proteins).
4. Wash cells using Buffer 1 as described in step 1 (2 X 5 mins). Resuspend in 1 ml Buffer 1.
5. Dilute NANOGOLD® ~ 50 times in Buffer 1 and add 30 ml to cells; incubate for 30 minutes with occasional shaking.
6. Wash cells in buffer 1 as described in step 1 (2 X 5 mins).
7. Fix cells and antibodies using a final concentration of 1% glutaraldehyde in PBS for 15 minutes. Then remove fixative by washing with buffer 1 (3 X 5 mins).
8. Rinse with deionized water (2 X 5 mins).
9. OPTIONAL (may reduce background): Rinse with 0.02 M sodium citrate buffer, pH 7.0 (3 X 5 mins).
10. Prepare HQ SILVER™ using equal amounts of the three components. Dispense initiator (A) into a clean tube or dish, add moderator (B), and mix thoroughly, then add activator (C) and mix thoroughly again to prepare the reagent. Develop specimen for 30 seconds - 4 minutes. More or less time can be used to control particle size and intensity of signal. A series of different development times should be tried, to find the optimum time for your experiment.
11. Rinse with deionized water (3 X 1 min).
12. Mount and stain as usual.

Negative staining may be used for electron microscopy of small structures or single molecules which are not embedded. Negative stain must be applied after the silver enhancement. Our NANOVAN™ negative stain is specially formulated for use with NANOGOLD® reagents; it is based on vanadium, which gives a lighter stain than uranium, lead or tungsten-based negative stains and allows easier visualization of NANOGOLD® particles with low silver enhancement.<sup>3</sup>

Buffer 1: 20 mM phosphate  
150 mM NaCl  
pH 7.4  
1 % BSA (bovine serum albumin)  
2 mM sodium azide (NaN<sub>3</sub>)

If a sensitive antigen is present, steps 1 and 2 may be omitted.

### Thin Sections

For the pre-embedding method, cells are labeled in suspension, as described above, and embedded after labeling.<sup>4,5</sup> Silver enhancement may be performed before or after embedding, but must be completed before treatment with any heavy metal staining reagent.

#### PROCEDURE FOR PRE-EMBEDDING METHOD:<sup>4</sup>

1. Incubate cells with 1 % bovine serum albumin in PBS at pH 7.4 (PBS-BSA) for 5 minutes; this blocks any non-specific protein binding sites and minimizes non-specific antibody binding.
2. Incubate with primary antibody, diluted at usual working concentration in PBS-BSA (30 mins - 1 hour, or usual time)
3. Rinse with PBS-BSA (3 X 1 min).
4. Incubate with NANOGOLD<sup>®</sup> reagent diluted 1/40 - 1/200 in PBS-BSA with 1 % normal serum from the same species as the NANOGOLD<sup>®</sup> reagent, for 10 minutes to 1 hour at room temperature.
5. Rinse with PBS-BSA (3 X 1 min), then PBS (3 X 1 min).
6. Postfix with 1 % glutaraldehyde in PBS (10 mins).
7. Rinse with deionized water (2 X 1 min).
8. OPTIONAL (may reduce background): Rinse with 0.02 M sodium citrate buffer, pH 7.0 (3 X 5 mins).
9. Prepare HQ SILVER<sup>™</sup> using equal amounts of the three components. Dispense initiator (A) into a clean tube or dish, add moderator (B), and mix thoroughly, then add activator (C) and mix thoroughly again to prepare the reagent. Develop specimen for 30 seconds - 4 minutes. More or less time can be used to control particle size and intensity of signal. A series of different development times should be tried, to find the optimum time for your experiment.
10. Rinse in deionized water for 5 minutes, twice.
11. Dehydrate and embed according to usual procedure. Use of a low-temperature resin (eg. Lowicryl) is recommended.
12. Stain (uranyl acetate, lead citrate or other positive staining reagent) if necessary before examination.

Alternatively, silver enhancement may be performed after embedding. Postfixing with osmium tetroxide or uranyl acetate may be carried out after silver enhancement is complete. 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.

#### PROCEDURE FOR POST-EMBEDDING METHOD:<sup>4,5</sup>

1. Prepare sections on plastic or carbon-coated nickel grid.
2. Incubate with 1 % solution of bovine serum albumin in PBS (PBS-BSA) for 5 minutes to block non-specific protein binding sites.
3. Incubate with primary antibody, diluted at usual working concentration in PBS-BSA (1 hour or usual time)
4. Rinse with PBS-BSA (3 X 1 min).
5. Incubate with NANOGOLD<sup>®</sup> reagent diluted 1/40 - 1/200 in PBS-BSA with 1 % normal serum from the same species as the NANOGOLD<sup>®</sup> reagent, for 10 minutes to 1 hour at room temperature.
6. Rinse with PBS (3 X 1 min).
7. Postfix with 1 % glutaraldehyde in PBS at room temperature (3 mins).
8. Rinse with deionized water (2 X 1 min).
9. OPTIONAL (may reduce background): Rinse with 0.02 M sodium citrate buffer, pH 7.0 (3 X 5 mins).
10. Prepare HQ SILVER<sup>™</sup> using equal amounts of the three components. Dispense initiator (A) into a clean tube or dish, add moderator (B), and mix thoroughly, then add activator (C) and mix thoroughly again to prepare the reagent. Develop specimen for 30 seconds - 4 minutes. More or less time can be used to control particle size and intensity of signal. A series of different development times should be tried, to find the optimum time for your experiment.
11. Rinse in deionized water for 5 minutes, twice.
12. If desired, contrast sections with uranyl acetate and/or lead citrate before examination.

**PBS Buffer:**

20 mM phosphate  
150 mM NaCl  
pH 7.4

**NOTE:** Treatment with osmium tetroxide followed by uranyl acetate staining can lead to much more drastic loss of the silver enhanced NANOGOLD<sup>®</sup> particles. This may be prevented by gold toning:<sup>6</sup>

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.

**HQ SILVER ENHANCEMENT FOR LIGHT MICROSCOPY**

**IMPORTANT:** solutions must be thawed and allowed to thermally equilibrate to room temperature before use to ensure uniform, reproducible results. 2 hours is recommended.

If aldehyde-containing reagents have been used for fixation, these must 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. HQ SILVER<sup>™</sup> is prepared immediately before use: equal amounts of each component (e.g. 0.5 mL of each) should be used. Mix initiator (solution A) and moderator (solution B) thoroughly, then add activator (solution C) and mix thoroughly again. The reagents are supplied in dropping bottles for easier dispensing of small amounts. The moderator and activator are viscous; mixing will be easier if the components are allowed to reach room temperature first. Development should be performed in a darkroom or dark box. NANOGOLD<sup>®</sup> will nucleate silver deposition resulting in a dark staining, depending on development time. Optimum results should be obtained using the buffers and washes specified in the instructions for NANOGOLD<sup>®</sup> reagents.

1. Spin cells onto slides using Cytospin, or use paraffin section.
2. Incubate with 1 % solution of bovine serum albumin in PBS (PBS-BSA) for 10 minutes to block non-specific protein binding sites.
3. Incubate with primary antibody, diluted at usual working concentration in PBS-BSA (1 hour or usual time)
4. Rinse with PBS-BSA (3 X 2 min).
5. Incubate with NANOGOLD<sup>®</sup> reagent diluted 1/40 - 1/200 in PBS-BSA with 1 % normal serum from the same species as the NANOGOLD<sup>®</sup> reagent, for 1 hour at room temperature.
6. Rinse with PBS (3 X 5 min).
7. Postfix with 1 % glutaraldehyde in PBS at room temperature (3 mins).
8. Rinse with deionized water (3 X 1 min).
9. OPTIONAL (may reduce background): Rinse with 0.02 M sodium citrate buffer, pH 7.0 (3 X 5 mins).
10. Prepare HQ SILVER<sup>™</sup> using equal amounts of the three components. Dispense initiator (A) into a clean tube or dish, add moderator (B), and mix thoroughly, then add activator (C) and mix thoroughly again to prepare the reagent. Develop specimen for 2 - 10 minutes. More or less time can be used to control particle size and intensity of signal. A series of different development times should be tried, to find the optimum time for your experiment; generally a shorter antibody incubation time will require a longer silver development time.
11. Rinse with deionized water (2 X 5 mins).
12. The specimen may now be stained if desired before examination, with usual reagents.

**PBS-BSA Buffer:**

20 mM phosphate  
 150 mM NaCl  
 pH 7.4  
 0.5% BSA  
 0.1% gelatin (high purity)  
*optional, may reduce background:*  
 0.5 M NaCl  
 0.05% Tween 20

**PBS Buffer:**

20 mM phosphate  
 150 mM NaCl  
 pH 7.4

To obtain an especially dark silver signal, the silver enhancement may be repeated with a freshly mixed portion of HQ SILVER™.

**HQ SILVER ENHANCEMENT FOR IMMUNOBLOTS**

**IMPORTANT:** solutions must be thawed and allowed to thermally equilibrate to room temperature before use to ensure uniform, reproducible results. 2 hours is recommended.

In most situations, LI SILVER is the reagent of choice for blotting applications; however, HQ SILVER will also give good results if it is required. The basic procedure for gold immunoblotting has been described by Moeremans et al,<sup>7</sup> which may be followed. For best results, the membrane should be hydrated before use by simmering in gently boiling water for 15 minutes. HQ SILVER™ is prepared immediately before use: equal amounts of each component (e.g. 2 mL of each) should be used. Mix initiator (solution A) and moderator (solution B) thoroughly, then add activator (solution C) and mix thoroughly again. The reagents are supplied in dropping bottles for easier dispensing of the same amounts. The moderator and activator are viscous; mixing will be easier if the components are allowed to reach room temperature first. Development should be performed in a darkroom or dark box. 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 antigen 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 primary antibody according to usual procedure (usually 1 or 2 hours).
4. Rinse with buffer 1 (3 X 10 mins).
5. Incubate with a 1/100 to 1/200 dilution of the NANOGOLD® reagent in buffer 2 for 2 hours at room temperature.
6. Rinse with buffer 3 (3 X 5 mins), then buffer 4 (2 X 5 mins).
7. OPTIONAL (may improve sensitivity): Postfix with glutaraldehyde, 1 % in buffer 4 (10 mins).
8. Rinse with deionized water (2 X 5 mins).
9. OPTIONAL (may reduce background): Rinse with 0.05 M EDTA at pH 4.5 (5 mins).
10. Prepare HQ SILVER™ using equal amounts of the three components. Dispense initiator (A) into a clean tube or dish, add moderator (B), and mix thoroughly, then add activator (C) and mix thoroughly again to prepare the reagent. Develop specimen for 5 - 10 minutes.
11. Rinse repeatedly with deionized water.

To obtain an especially dark silver signal, the silver enhancement may be repeated with a freshly mixed portion of HQ SILVER™.

**Buffer 1:** 20 mM phosphate  
 150 mM NaCl  
 pH 7.4  
 4% BSA (bovine serum albumin)  
 2 mM sodium azide (NaN<sub>3</sub>)

**Buffer 2:** 20 mM phosphate  
 150 mM NaCl  
 pH 7.4  
 0.8% BSA  
 1% normal serum; use serum of the host animal  
 for the NANOGOLD® antibody  
 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<sub>3</sub>)

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

Other procedures may be used; for example the NANOGOLD<sup>®</sup> reagent may be used as a tertiary labeled antibody, or a custom NANOGOLD<sup>®</sup> conjugate may be the primary antibody. If additional antibody incubation steps are used, rinse with buffer 3 (3 X 10 mins) after incubation.

## **REFERENCES**

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