

LI SILVER™



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PRODUCT INFORMATION

LI SILVER™ ENHANCEMENT KIT (125 mL)

Product Name: LI SILVER
Catalog Number: 2013
Appearance: Colorless solutions
Revision: 1.5 (January 2009)

GENERAL INFORMATION

LI SILVER™ is a convenient, light-insensitive silver enhancement system for use with NANOGOLD® reagents, which may be used for EM or light microscopy or to visualize NANOGOLD® on blots. LI SILVER consists of 125 mL ready-to-use enhancer and 125 mL ready-to-use initiator; the reagent is formed by combining equal volumes of these solutions. The mixture is usable only for a defined time period, as given below, and should therefore be prepared immediately before use. LI SILVER is nucleated quickly by NANOGOLD® particles, resulting in the precipitation of metallic silver and the formation of a dark brown to black signal. The system has markedly delayed self-nucleation resulting in high contrast and very low background and non-specific staining.

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 45 minutes; at 20°C, the solution is stable for at least 40 minutes, and at 24°C for at least 35 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 enhancer and initiator solutions at 2 - 8°C. DO NOT FREEZE. Do not expose to extreme heat or light. Avoid contact with metallic objects, since these can induce silver precipitation. Avoid cross-contamination of the enhancer and initiator solutions. Avoid skin contact: the silver enhancement reagents will stain skin.

Samples must be rinsed with deionized water before silver enhancement. This is because the reagent contains silver ions in solution, which react to form a precipitate with chloride, phosphate and other anions which are components of buffer solutions.

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.

Note: All LI SILVER™ solutions should be equilibrated to room temperature prior to the enhancement procedure.

LI SILVER ENHANCEMENT FOR EM

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 must 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. 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).

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 10⁷ 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 minutes). 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 minutes). 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 minutes).
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 minutes).
8. Rinse with deionized water (2 X 5 minutes).
9. Develop with freshly mixed LI SILVER[™] enhancer for 1 - 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 with deionized water (3 X 1 minute).
11. 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.²

Buffer 1: 20 mM phosphate
150 mM NaCl
pH 7.4
1 % BSA (bovine serum albumin)
2 mM sodium azide (NaN₃)

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. 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:³

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 minutes - 1 hour, or usual time)
3. Rinse with PBS-BSA (3 X 1 minute).
4. Incubate with NANOGOLD[®] reagent diluted 1/40 - 1/200 in PBS-BSA with 1 % normal serum from the same species as the NANOGOLD[®] reagent, for 10 minutes to 1 hour at room temperature.
5. Rinse with PBS-BSA (3 X 1 min), then PBS (3 X 1 minute).
6. Postfix with 1 % glutaraldehyde in PBS (10 minutes).
7. Rinse with deionized water (2 X 1 minute).
8. Develop specimen with freshly mixed LI SILVER[™] enhancer for 1 - 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.
9. Rinse in deionized water for 5 minutes, twice.
10. Dehydrate and embed according to usual procedure. Use of a low-temperature resin (e.g. Lowicryl) is recommended.
11. 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:^{3,4}

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 minute).
5. Incubate with NANOGOLD[®] reagent diluted 1/40 - 1/200 in PBS-BSA with 1 % normal serum from the same species as the NANOGOLD[®] reagent, for 10 minutes to 1 hour at room temperature.
6. Rinse with PBS (3 X 1 minute).
7. Postfix with 1 % glutaraldehyde in PBS at room temperature (3 minutes).
8. Rinse with deionized water (2 X 1 minute).
9. Develop specimen with freshly mixed LI SILVER[™] enhancer for 1 - 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. If desired, contrast sections with uranyl acetate and/or lead citrate before examination.

PBS Buffer:

20 mM phosphate
150 mM NaCl
pH 7.4

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.⁵

1. After silver enhancement, wash thoroughly with deionized 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.

LI SILVER ENHANCEMENT FOR LIGHT MICROSCOPY

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. To prepare the developer, mix equal amounts of the enhancer and initiator immediately before use. NANOGOLD® will nucleate silver deposition resulting in a dark staining depending on development time. Additional steps, such as postfixing, may be used as required. Optimum results should be obtained using the buffers and washes specified in the instructions for the NANOGOLD® 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 minutes).
5. Incubate with NANOGOLD® reagent diluted 1/40 - 1/200 in PBS-BSA with 1 % normal serum from the same species as the NANOGOLD® reagent, for 1 hour at room temperature.
6. Rinse with PBS (3 X 5 minute).
7. Postfix with 1 % glutaraldehyde in PBS at room temperature (3 minutes).
8. Rinse with deionized water (3 X 1 minute).
9. Develop specimen with freshly mixed LI SILVER™ enhancer for 5-20 minutes. More or less time can be used to control intensity of signal. A series of different development times may be used, to find the optimum enhancement for your experiment; generally a shorter antibody incubation time will require a longer silver development time.
10. Rinse with deionized water (2 X 5 minutes).
11. 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 LI SILVER™.

LI SILVER ENHANCEMENT FOR IMMUNOBLOTS

The basic procedure for gold immunoblotting has been described by Moeremans et al,⁶ which may be followed. For best results, the membrane should be hydrated before use by simmering in gently boiling water for 15 minutes. 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 $\text{pg} / \mu\text{L}$.
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 minutes).
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 minutes), then buffer 4 (2 X 5 minutes).
7. OPTIONAL (may improve sensitivity): Postfix with glutaraldehyde, 1 % in buffer 4 (10 minutes).
8. Rinse with deionized water (2 X 5 minutes).
9. OPTIONAL (may reduce background): Rinse with 0.05 M EDTA at pH 4.5 (5 minutes).
10. Develop with freshly mixed LI SILVER[™] enhancer for 20-25 minutes, twice. Rinse thoroughly with deionized water between developments to remove all the reagent.
11. Rinse repeatedly with deionized water.

Buffer 1: 20 mM phosphate
150 mM NaCl
pH 7.4
4% BSA (bovine serum albumin)
2 mM sodium azide (NaN_3)

Buffer 3: 20 mM phosphate
150 mM NaCl
pH 7.4
0.8% BSA (bovine serum albumin)
2 mM sodium azide (NaN_3)

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

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

REFERENCES

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Technical assistance available.

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