Revised: 09–May–2001

LI Silver Enhancement Kit (L-24919)

Quick Facts

Storage upon receipt:

- 4°C
- Do not freeze
- Protect from light

Introduction

LI Silver Enhancement Kit is a convenient, light-insensitive silver enhancement system for use with our NANOGOLD® reagents, which can be used for EM or light microscopy or to visualize NANOGOLD on blots.[1] 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 (see Figure 1). The system has markedly delayed self-nucleation, resulting in high contrast and very low backgrounds.

Gold particles in the presence of silver (I) ions and a reducing agent will act as catalysts to reduce silver (I) ions to metallic silver. The silver is deposited onto the gold, enlarging the particles to between 30 and 100 nm in diameter. Tissue or blots stained with colloidal gold are "developed" by this autometallographic procedure to give black staining which can be seen in the light microscope. This method known as immunogold silver staining (IGSS) has been widely used with the NANOGOLD cluster probe; it is one of the most sensitive immunodetection systems available, similar to chemiluminescence and radionuclide labeling. In blots, as little as 0.1 pg of a target IgG has been detected using a NANOGOLD Fab′ probe.[2] NANOGOLD streptavidin has proven to be highly sensitive in detecting biotinylated nucleic acid probes in *in situ* hybridization studies.

Molecular Probes' LI Silver Enhancement Kit is ideal for use with our NANOGOLD reagents. The advantages of the LI Silver Enhancement Kit are:

- High contrast signal for easy light microscope and immunoblot visibility
- Lower background than other commercial developers
- High sensitivity
- Light insensitive: observe development under normal lighting
- Slow development (10–30 minutes) for precise monitoring
- Compatible with all immunogold reagents
- Best for light microscopy, gels and Western blots

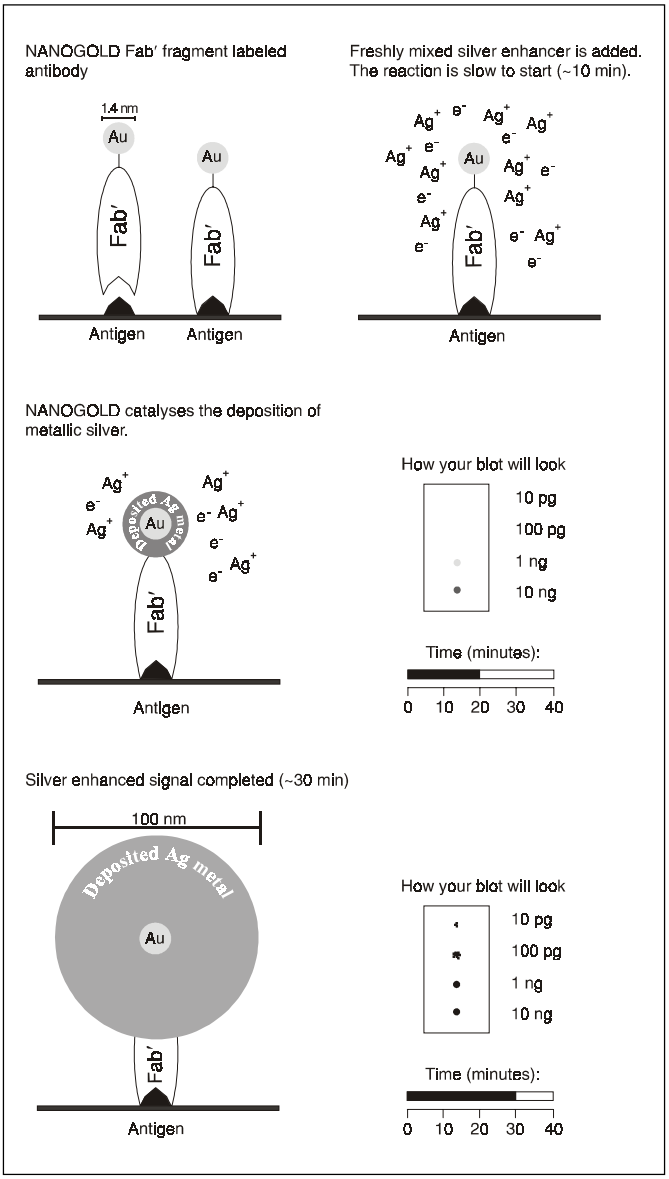

Figure 1. *Diagram illustrating the silver enhancement reaction. NANOGOLD-labeled antibodies bind to the antigen and silver ions in solution nucleate around the gold particles, precipitating as silver metal. The silver-enhanced particle grows in size with time: short development is used for electron microscopy, intermediate time for light microscopy and longer times for blots and gels.*

Materials

Contents

- **Enhancer reagent** (Component A), 250 mL
- **Initiator reagent** (Component B), 250 mL

Combining equal volumes of these solutions forms the developer solution. The mixture is usable for only a defined time period, as given below, and should be prepared immediately before use.

Storage

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.

WARNING: Avoid skin contact — the silver enhancement reagents will stain skin.

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

Application

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.

Staining Protocols

LI Silver Enhancement for EM

If specimens are to be embedded, silver enhancement is usually performed after embedding, although it may be done first.[3] Silver enhancement 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 nonspecific staining. With NANOGOLD reagents, low-temperature resins like Lowicryl (Electron Microscopy Sciences, Fort Washington, PA) should be used and the specimens kept at or below room temperature until the silver development has been completed. LI silver enhancement of NANOGOLD is recommended for applications where osmium tetroxide, lead citrate or uranyl acetate stains are also 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. Quenching may be achieved by incubating the specimens for 5 minutes in a 50 mM glycine solution in phosphate-buffered saline (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 LI 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 nonspecific staining. To prepare the developer, mix equal amounts of the enhancer (Component A) and initiator (Component B) 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).

LI Silver Enhancement for Light Microscopy

If aldehyde-containing reagents have been used for fixation, these must be quenched before labeling. Quenching 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 (Component A) and initiator (Component B) immediately before use. NANOGOLD will nucleate silver deposition resulting in a dark staining depending on development time. Additional steps, such as post-fixing, may be used as required.

1.1 Develop the specimen with freshly mixed developer for 5–20 minutes. More or less time can be used to control the 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.

1.2 Stop the reaction by rinsing twice with deionized water for 5 minutes each.

1.3 The specimen may now be stained with additional reagents, if desired, before examination.

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

LI Silver Enhancement for Immunoblots[4]

The membrane should be hydrated before use by simmering it 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:

2.1 Rinse the immunoblot twice with deionized water for 5 minutes each.

2.2 OPTIONAL (may reduce background): Rinse the blot with 0.05 M EDTA at pH 4.5 for 5 minutes.

2.3 Develop the blot with freshly mixed LI silver developer for 20–25 minutes. Repeat.

2.4 Rinse repeatedly with deionized water.

Detection of NANOGOLD-Labeled Molecules on Gels

NANOGOLD particles (1.4 nm diameter) may be developed with silver so that they become visible to the naked eye, thus amplifying the signal thousands of times. If you have used NANOGOLD mono(sulfosuccinimidyl ester) or NANOGOLD monomaleimide to label a protein or other molecule, these may then easily be analyzed and detected on gels using silver enhancement.

3.1 After labeling with NANOGOLD, remove unbound gold particles by column chromatography, sucrose gradient or other purification means. Leaving excess free NANOGOLD in the sample will interfere with the intended gel staining.

3.2 Run the gel as usual with two precautions:

- NANOGOLD is degraded by β-mercaptoethanol (or DTT), so the sample must not be mixed with a reducing agent, i.e., a nonreducing gel must be run. Normal concentrations of other ingredients (SDS, etc.) are acceptable.

- The sample must not be heated. Frequently samples are boiled in SDS before loading on the gel. Since NANOGOLD is degraded above 50°C, heating is not recommended. This is usually not a limitation, since normal gel patterns are obtained with most samples without heating.

3.3 The gel may be electrotransferred to nitrocellulose, if desired, although this is not necessary.

3.4 Rinse the gel with several changes of deionized water. Since the silver developer is precipitated by halides, traces of NaCl must be removed.

3.5 Place the gel or blot in a suitable dish and apply enough freshly prepared LI silver developer to cover the gel. Do not use the usual silver stains for gels because these stains are quite different from LI silver enhancement and do not effectively develop NANOGOLD.

3.6 Watch the development of band(s), which should appear brown-black. Aggregates with gold that did not enter the gel or small amounts of free gold may give background staining. Usual development time is 1–5 minutes. Extensive development time (>30 min) will lead to some nonspecific background staining due to self-nucleation of the developer.

3.7 When optimal staining is reached, stop the development by rinsing in deionized water. The final stained gel is now a permanent record.

3.8 For comparison and visualization of all bands, run a duplicate gel and stain with one of Molecular Probes' SYPRO® protein gel stains, Coomassie™ Blue or a gel silver stain.

3.9 A NANOGOLD-labeled molecule typically runs ~15,000 MW higher on the gel due to the added weight of the NANOGOLD particle (~15,000). Thus labeled and unlabeled molecules are separated, and their proportion may be estimated by usual gel stains (e.g., SYPRO stain), which show total protein content.

References

1. *Immunogold-Silver Staining: Principles, Methods and Applications*, CRC Press (1995) pp. 71–96; **2.** J Histochem Cytochem 40, 177–184 (1992); **3.** *Colloidal Gold: Principles, Methods and Applications, vol. 1*, Hayat M.A., Ed., Academic Press (1989) pp. 421–425; **4.** J Immunol Methods 74, 353 (1984).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
L-24919	LI Silver (LIS) Enhancement Kit	1 kit

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

Molecular Probes, Inc.
PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 ● Fax: (541) 344-6504

Customer Service: 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 ● Fax: (541) 344-6504 ● order@probes.com

Toll-Free Ordering for USA and Canada:
Order Phone: (800) 438-2209 ● Order Fax: (800) 438-0228

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 ● Fax: (541) 465-4593 ● tech@probes.com

Molecular Probes Europe BV
PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 ● Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 ● Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 ● Fax: +31-71-5241883
eurotech@probes.nl

Molecular Probes' products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

The LI Silver Enhancement Kit is prepared for Molecular Probes by Nanoprobes, Inc.