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ProductInformation

Silver Enhancer Kit

Catalog Number **SE100**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Colloidal gold-protein complexes are used as probes in electron microscopy, light microscopy, and blotting procedures. The gold label is a discrete, electron-dense, non-fading, red-colored marker capable of strong secondary electron emission. It requires no additional processing for detection, but in certain applications the signal can be dramatically intensified by reaction with silver.

The Silver Enhancer Kit enlarges colloidal gold labels, normally visible only at the electron microscope level, by precipitation of metallic silver. This gives high-contrast signals visible by light microscopy. A darkroom is not necessary for this procedure, as the silver enhancer working solution is light stable for 20–30 minutes under ordinary laboratory lighting.

Solution A and Solution B are mixed 1:1 immediately before use and applied to the slide holding the gold-labeled section. After 5–10 minutes, the slide is rinsed with distilled water, fixed for 2–3 minutes in a sodium thiosulfate solution, and rinsed again. The section is then ready for counterstaining and mounting as required.

Reagents

Sufficient reagents are provided for staining and fixing at least 100 slides.

Silver Enhancer Solution A Catalog Number S5020	100 ml
Silver Enhancer Solution B Catalog Number S5145	100 ml
Sodium thiosulfate, pentahydrate Catalog Number S8503	500 g

Kit reagents are available individually.

Reagents and Equipment Required but Not Provided

(Catalog Numbers given where appropriate)

- 2.5% Glutaraldehyde Solution
- Tris Buffered Saline (TBS), pH 7.7
- Phosphate Buffered Saline (PBS), pH 7.4
- Normal Goat Serum (Catalog Number G6767) **or** Normal Rabbit Serum (Catalog Number R4505)

Optional:

- Bovine Serum Albumin (Catalog Numbers A7030 or A7638) - May improve results when using primary or secondary antibodies in staining procedure.
- Iodine (Catalog Number I3380) and potassium iodide (Catalog Number 207969) - For treatment of tissue sections with Lugol Solution
- Trypsin (Catalog Number T7409) and calcium chloride (Catalog Number C3881) - For treatment of tissue sections with proteolytic enzymes.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Avoid contact with skin. Solutions A and B may stain the skin brown both before and after mixing.

Preparation Instructions
Silver Enhancer Mixture – Just prior to use, mix equal volumes of Silver Enhancer Solutions A and B. Approximately 2 ml of the mixture is required per slide for individual sections. Be careful not to interchange the bottle caps. Accidental cross-contamination of Solutions A and B should be avoided.

2.5% Sodium Thiosulfate Solution – 3.9 g of Sodium thiosulfate, pentahydrate (Catalog Number S8503) in a final volume of 100 ml of distilled water,

Tris Buffered Saline (TBS), pH 7.7 – Add 2.4 g of Trizma® Base (Catalog Number T1503) and 8.9 g of sodium chloride (Catalog Number S9888) to 1,000 ml of distilled water and adjust pH as required with 1 M HCl.

2.5% Glutaraldehyde Solution - A 10-fold dilution of 25% Glutaraldehyde, Catalog Number G5882 may be used.

Lugol Solution- 1% iodine in 2% aqueous potassium iodide

Storage/Stability
Silver Enhancer Solutions A and B are mildly light and heat sensitive. Storage in the dark at 2–8 °C is recommended.

A darkroom is not necessary for this procedure, as the Silver Enhancer Mixture is light stable for 20–30 minutes under ordinary laboratory lighting. Direct sunlight should be avoided.

Procedures
All glassware should be thoroughly rinsed with distilled water, particularly if chromic acid or detergents have been used.

Do not use metal forceps to hold slides, since heavy metals catalyze the silver precipitation reaction.

<u>Specimen Preparation</u>

A. Paraffin Sections - Paraffin wax embedded sections should be dewaxed and rehydrated. If treatment with Lugol Solution or enzymatic digestion is not required, proceed to Staining Procedure.

B. Frozen, Cryostat Sections - Treatment with Lugol Solution is not usually necessary. However, if a specific application requires either Lugol Solution treatment or enzymatic digestion, follow procedures C or D. Otherwise, proceed to Staining Procedure.

C. Treatment With Lugol Solution - Treating tissue sections with Lugol Solution can increase sensitivity, allowing for higher dilutions of primary antibody. Lugol Solution treatment is usually not necessary if enzymatic digestion is carried out.

    1. Immerse slides in distilled water.
    2. Then immerse slides in Lugol Solution for 5 minutes.
    3. Rinse well with distilled water.
    4. Immerse slides in 2.5% Sodium Thiosulfate Solution for 2–3 minutes or until colorless.
    5. Rinse well with gently running tap water for 5 minutes.
    6. Transfer slides to distilled water.
    7. Proceed to Staining Procedure.

D. Enzymatic Digestion - Treatment of tissue sections with proteolytic enzymes is sometimes necessary for particular applications.

    1. Transfer section to Tris Buffered Saline (TBS), pH 7.7. (See Preparation Instructions)
    2. Incubate section in 0.1% Trypsin in TBS, pH 7.7, with 4 mM $CaCl_2$ for 10–12 minutes at 37 °C (30 minutes at 20 °C).
    3. Rinse with gently running tap water for 5 minutes.
    4. Transfer to distilled water.
    5. Proceed to Staining Procedure.

Staining Procedure

1. Rinse section in required buffer, either as indicated by the manufacturer of the primary antibody, or as preferred (e.g., TBS or PBS).
2. Dry the area around the specimen with tissue. For convenience, score the slide with a diamond, marking the specimen area for subsequent washing steps.
3. Incubate with blocking buffer for 5–15 minutes using 5% normal serum from the same species as the secondary antibody-gold conjugate, diluted in the chosen buffer.
4. Remove excess blocking buffer by wiping around the specimen with tissue.
5. Incubate with primary antibody using dilutions and incubation times recommended by the manufacturer or as determined empirically. As a guide, dilutions of at least 1:100 and an incubation time of 1 hour are appropriate. The IGSS technique is very sensitive and antibody dilutions of up to 1:1,000 may often be used. Therefore, a series of primary antibody dilutions is recommended. At this stage, the section may be incubated in primary antibody overnight. Place the slide in a humidified chamber. Do not allow sections to dry out during staining procedures.
6. Rinse section in buffer for 3–5 minutes. Remove excess buffer by wiping around the section with tissue.
   Note: Addition of 0.1% Bovine Serum Albumin and/or 1% normal serum of the same species as the colloidal gold conjugate to the wash buffer for steps 6 and 8 may yield improved results when using primary or secondary antibodies in staining procedures.
7. Apply the appropriate immunogold reagent, ~100 µl per slide is required, and incubate one hour. A 1:50 dilution in buffer of the secondary antibody-colloidal gold conjugate is usually sufficient, although it is often possible to use a greater dilution, as determined empirically.
8. Rinse section again in buffer for 3–5 minutes and remove excess buffer with tissue.
9. Fix specimen in 2.5% glutaraldehyde solution in buffer for at least 15 minutes. (See Preparation Instructions). Sections may be left overnight at this stage if necessary.
10. Thoroughly rinse section in distilled water to remove all traces of chlorides, buffer salts, etc. Leave slides immersed in distilled water in readiness for silver enhancement.

Silver Enhancement

This Silver Enhancement procedure is applicable to other colloidal gold labels, e.g., lectins, streptavidin, etc. For these applications, follow an appropriate staining procedure. Then continue with the Staining Procedure, step 8, before using Silver Enhancement.

It is not necessary to use a darkroom. The Silver Enhancer Mixture is stable for 20–30 minutes at 20 °C under normal laboratory lighting, longer in subdued light. Direct sunlight should be avoided.

1. Wipe off excess water with tissue. (See Staining Procedure, step 10)
2. Add enough Silver Enhancer Mixture to cover the specimen, ~ 2 ml. Develop the section in the enhancer until the desired stain intensity is reached. Typical enhancement time at 20 °C is 5–10 minutes (depending on the application), increasing at lower temperatures and decreasing at higher temperatures. For research applications, or when using a colloidal gold conjugate for the first time, monitor the enhancement under a light microscope. Stop the reaction when the desired stain intensity is reached.
3. Rinse section in distilled water to remove the Silver Enhancer Mixture.
4. Fix by immersing in 2.5% Sodium Thiosulfate Solution (see Preparation Instructions) for 2–3 minutes. Do not leave in fixer for more than 5 minutes.
5. Wash section thoroughly in distilled water.
6. Counterstain and mount as desired.

References

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