SIGMA CHEMICAL COMPANY
P.O. BOX 14508, ST. LOUIS, MO 63178 USA

Product No. SE-100

# SILVER ENHANCER KIT

For Use in Immunogold Silver Staining (IGSS)

Issued January 1989 Technical Bulletin No. SE-1

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**SIGMA CHEMIE GmbH** Grünwalder Weg 30 • D-8024 Deisenhofen • W. Germany • Telephone: **089/61301 - 0.**
**SIGMA CHIMIE S.a.r.l.** •L'Isle d'Abeau Chesnes,•B.P. 701, 38297•La Verpillière, France•Telephone **74 95 63 91**

## SUMMARY

Colloidal gold-protein complexes have gained wide use 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.

On addition of Sigma Silver Enhancer, precipitation of metallic silver occurs, which enlarges colloidal gold labels normally visible only at the electron microscope level, yielding high-contrast signals visible by light microscopy.

The Silver Enhancer Kit consists of a silver salt (Solution A), an initiator (Solution B), a fixer (sodium thiosulfate), and Technical Bulletin SE-1.

Solutions A and B are mixed 1:1 immediately before use and applied to a slide holding the gold-labeled section. After 5-10 minutes (depending on the application), the slide is rinsed, fixed for 2-3 minutes in a sodium thiosulfate solution and rinsed again. After counterstaining and mounting, the section is ready for light microscopy.

Silver Enhancer reagents are stable when stored in the dark at 4°C. The mixed enhancer solution is stable for 20-30 minutes under ordinary laboratory lighting. A darkroom is not necessary.

## REAGENTS

Sigma Silver Enhancer Kit, when used according to directions, is sufficient for staining and fixing at least 100 slides. Reagents include:

| | |
|---|---|
| **Silver Enhancer Solution A** | 100 ml |
| **Silver Enhancer Solution B** | 100 ml |
| **Sodium Thiosulfate Pentahydrate** | 500 g |

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

**Required But Not Provided:**

Glutaraldehyde, 2.5% solution
(A 1:10 dilution of 25% Glutaraldehyde, Sigma Product No. G 5882, may be used.)

Tris Buffered Saline (TBS), pH 7.6
2.4 g Trizma Base (Sigma Product No. T 1503)
8.9 g Sodium Chloride (Sigma Product No. S 9888)
1000 ml distilled water
adjust pH as required with 1M HCl

Phosphate Buffered Saline (PBS), pH 7.4
(Sigma Product No. 1000-3)

Either Normal Goat Serum (Sigma Product No. G 6767) or
Normal Rabbit Serum (Sigma Product No. R 4505)
(See Tissue Culture/Sera section of Sigma Catalog.)

**Optional Reagents:**

Bovine Serum Albumin (Sigma Product No. A 7030 or A 7638)
(May improve results when using primary or secondary antibodies in staining procedure.)

Iodine (Sigma Product No. I 3380) and
Potassium Iodide (Sigma Product No. P 8256)
(For treatment of tissue sections with Lugol's Iodine.)

Trypsin (Sigma Product No. T 8128 or T 0134) and
Calcium Chloride (Sigma Product No. C 3881)
(For treatment of tissue sections with proteolytic enzymes.

## STORAGE AND STABILITY

Silver Enhancer Solutions A and B are mildly light and heat sensitive. Storage in the dark at 4°C is recommended.

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

Accidental cross-contamination of Solutions A and B should be avoided.

Heavy metals catalyze the silver precipitation reaction. Do not use metal forceps to hold slides.

# SPECIMEN PREPARATION

**Paraffin Sections:**

Paraffin wax embedded sections should be dewaxed and rehydrated. If treatment with Lugol's Iodine or enzymatic digestion is not required, proceed to **STAINING PROCEDURE.**

**Frozen, Cryostat Sections:**

Treatment with Lugol's Iodine is not usually necessary. However, if a specific application requires either Lugol's Iodine treatment or enzymatic digestion, follow the procedures described below. Otherwise, proceed to **STAINING PROCEDURE.**

**Treatment With Lugol's Iodine:**

Treating tissue sections with Lugol's Iodine can increase sensitivity, allowing for higher dilutions of primary antibody. Lugol's Iodine treatment is usually not necessary if enzymatic digestion is carried out.

1. Immerse slides in distilled water.
2. Then immerse slides in Lugol's Iodine (1% iodine in 2% aqueous potassium iodide) for 5 minutes.
3. Rinse well with distilled water.
4. Immerse slides in 2.5% aqueous 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.**

**Enzymatic Digestion:**

Treatment of tissue sections with proteolytic enzymes is sometimes necessary for particular applications.

1. Transfer section to Tris Buffered Saline (TBS), pH 7.8. (See **REAGENTS.**)
2. Incubate section in either 0.1% Trypsin (Sigma Product No. T 8128) or in 0.01% Trypsin (Sigma Product No. T 0134), dissolved in TBS containing 0.1% calcium chloride for approximately 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). (See **REAGENTS.**)
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 that of the second 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/supplier 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 1:1000 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.
7. Apply the appropriate immunogold reagent; approximately 100 $\mu$l per slide is required. Incubate one hour. A 1:50 dilution in buffer of the Sigma 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 **REAGENTS.**) 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.

NOTE: Addition of 0.1% Bovine Serum Albumin (see **REAGENTS**) and/or 1% normal serum of the same species as the colloidal gold conjugate to the wash buffer for steps 6-8 may yield improved results when using primary or secondary antibodies in staining procedures.

# SILVER ENHANCEMENT

It is not necessary to use a darkroom. The mixture of Enhancer Solutions A and B 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. Mix equal volumes of Sigma 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 or cross-contaminate the solutions. Do not use metal forceps to hold slides, since heavy metals catalyze the silver precipitation reaction.
3. Add enough Silver Enhancer mixture to cover the specimen. Develop the section in the enhancer until the desired stain intensity is reached. Typical enhancement time at 20°C is approximately 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.
4. Rinse section in distilled water to remove the enhancer.
5. Fix by immersing in 2.5% aqueous sodium thiosulfate (see **REAGENTS**) for 2-3 minutes. Do not leave in fixer for more than 5 minutes.
6. Wash section thoroughly in distilled water.
7. Counterstain and mount as desired.

**Other Applications:**

Silver Enhancement as described above 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 the Silver Enhancer.

**For a complete listing of Gold Colloid Conjugates available from Sigma, see pages 675-678 of the 1989 Sigma Catalog.**

# REFERENCES


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| Product No. | | Quantity |
|---|---|---|
| **SE-100** | **SILVER ENHANCER KIT** | |
| [O-5°C] | Solution A | 100 ml |
| | Solution B | 100 ml |
| | Sodium Thiosulfate Pentahydrate | 500 g |
| | Technical Bulletin No. SE-1 | |
| | Reagents are sufficient to stain and fix at least 100 slides | |

Kit reagents are available individually:

| | | |
|---|---|---|
| **S 5020** | **Silver Enhancer Solution A** | 100 ml |
| [O-5°C] | | 500 ml |
| **S 5145** | **Silver Enhancer Solution B** | 100 ml |
| [O-5°C] | | 500 ml |
| **S 8503** | **Sodium Thiosulfate Pentahydrate** (pfs) | 500 g |
| [RT] | | 2.5 kg |