# SILVER ENHANCEMENT KIT

(Cat. No.: SET-10)

## **INTENDED USE**

To be used with Colloidal Gold Particle labeled reagents to enhance sensitivity.

# SUMMARY AND PRINCIPLES OF PROCEDURES

The Silver Enhancement technique is used to amplify the pink-red stain obtained when using Colloidal Gold labeled glycoproteins, lectins, and immunoreagents. The Colloidal Gold particles acts as a catalyst for the deposition of metallic silver. This deposition changes the characteristic pink stain to a permanent black stain which can be several orders of magnitude more sensitive than using the Colloidal Gold alone. This permits detection of latent gold staining which gives extraordinarily high sensitivity. The photochemical reaction generally takes less than ten minutes.

# STORAGE CONDITIONS

All reagents supplied in this kit should be stored at room temperature.

# SAMPLE PROCESSING

After tissue section or nitrocellulose has been labeled with Colloidal Gold particle reagent, wash with distilled water to remove any chloride ions that may react with silver reagents.

# **RECOMMENDED PROCEDURE**

The reaction is light sensitive. Work in a dark room or under dim light conditions. Prepare enough staining reagent to ensure complete immersion of sample. The procedure detailed below should prepare 25ml of staining reagent.

- 1. Reconstitute one vial each of REAGENT 1 and REAGENT 2 according to label instructions. Mix contents of both vials together.
- 2. IMMEDIATELY PRIOR TO USE IN A DARKROOM reconstitute one vial of REAGENT 3 according to label instructions. Add this solution to solution prepared in step 1.
- Completely immerse tissue sections or nitrocellulose sheets for 2-3 minutes in solution prepared in step 2. If longer incubation times are required, rinse sections or nitrocellulose sheets with distilled water and place a second time in a fresh solution of REAGENTS 1, 2, and 3.
- 4. Dilute REAGENT 4 according to label instructions. Use same volume of diluted REAGENT 4 as was used in step above. Place sample in this solution for 2 minutes.
- 5. To prepare 25ml fixative add 3.125 ml of REAGENT 5A to 12.5ml tap water at 16-27°C. With rapid agitation add 0.35ml of REAGENT 5B (CAUTION: CONTAINS SULFURIC ACID). Add water to give a final volume of 25ml. Use same volume of fixative as was used in previous steps. Place sample in fixative for 2 minutes.

6. Rinse under rooming tap water for 15 minutes and air dry (nitrocellulose sheets) or dehydrate auf anount dissue sections).

# MATERIALS SUPPLIED

REAGENT 1: Buffer solution
REAGENT 2: Reducing agents (It is recommended that this reagent be Millipore filtered but is not required to be sterile filtered)
REAGENT 3: Silver lactate
REAGENT 4: Acetic acid
REAGENT 5: Fixative (solutions 5A and 5B are both required).

# MATERIALS NEEDED BUT NOT SUPPLIED

Nitrocellulose sheets Tissue section

# LIMITATIONS OF PROCEDURE

Solutions must be used IMMEDIATELY after preparation. DO NOT RE-USE reagents after staining. REAGENT 3 is light sensitive. When using under dim light conditions it is recommended that the reaction container be covered with foil or use a black box to protect light sensitive reagents.

# **TROUBLE SHOOTING GUIDE**

PROBLEM	CAUSE	SOLUTION
Low Sensitivity	<ol> <li>Insufficient Gold labeling.</li> <li>Active-SH groups on tissue may reduce Colloidal Gold.</li> <li>Insufficient incubation time with Silver Enhancement Reagents.</li> </ol>	<ol> <li>a) Increase incubation time.</li> <li>b) Increase concentration of Gold Labeled Reagents.</li> <li>2. Block Sulfhydryl groups with Lugol's iodine.</li> <li>3. Repeat incubation a second time with freshly prepared reagents.</li> </ol>
High Background	<ol> <li>Overexposure to reagents.</li> <li>Overexposure to light.</li> <li>Tissue section or nitrocellulose only partially immersed in solution.</li> </ol>	<ol> <li>Decrease exposure time to Gold Labeled Reagents.</li> <li>Cover specimen with foil or work in a dark room.</li> <li>Completely cover sample with Silver Enhancement Reagents.</li> </ol>

# REFERENCES

- 1) Holgate, C.S. (1983). J. Histochem. Cytochem. 31: 938-944.
- 2) Danscher (1981). Histochemistry. 71: 81-88
- 3) Danscher and Norgaard (1983). J. Histochem. Cytochem. 31: 1394.
- 4) Springall et al. (1984). Histochemistry 81:403
- 5) Manigley and Roth, J. (1985). Histochem. Cytochem. 33:1247-1251.
- 6) Binder et al. (1986). J. Cell Biol. 102: 1646.

# **EY** LABORATORIES, INC.

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Tel: 650-342-3296 Fax: 650-342-2648 Orders: 1-800-821-0044 (Outside CA only)

## **MATERIAL SAFETY DATA SHEET**

Effective Date: March 31, 2006 Revision 4 Page 1 of 2

#### **PRODUCT IDENTIFICATION**

- Name: Colloidal gold and colloidal silver labeled proteins, enzymes, and ligands.
- Catalog G-2 to G-40, XGP-2, GP-01 to GP-8006, GAP-01, FGP-01, HGP-01, Number(s): GP-01, TGP-01, CCG-0001 to CCG-1018, GA-02, GAA-02, GAB-01 to GAB-02, FGA-02, HGA-02, GB-01 to GB-02, CE-01 to GE-03, GH-01 to GH-02, GM-01 to GM-2701, GAF-001 to GAF-2404, SA-02, SB-01, SH-01, SP-01 to SP-014, SET-10.
- Formula: Complex polypeptides, enzymes, lectins, antibodies, and ligands coupled to colloidal gold or silver particles. Also, unconjugated colloidal gold particles.
- Synonyms: Protein A, Horseradish Peroxidase, Strept. Avidin, D-Biotin, Purified Antibodies, Bovine Serum Albumin, Fetuin, Ovomucoid, RNase, DNase I, Alkaline Phosphatase, Protein G, Monoclonal Antibodies, Lectins, Neoglycoproteins, Adriamycin, and Neomycin coupled to colloidal gold particles or silver colloidal particles, silver lactate, acetic acid.

#### **EMERGENCY INFORMATION**

EY Laboratories, Inc. 107 North Amphlett Blvd. San Mateo, CA 94401

# EMERGENCY PHONE: 650-342-3296

#### HAZARDOUS COMPONENTS

Specific protein or ligand or chemicals as listed on the vial label. These solutions contain less than 0.1mg per ml. Biological activity of these proteins will vary. Although these materials are not generally considered to be hazardous they may cause allergic responses in sensitive individuals if inhaled or allowed to contact skin. Adriamycin and Neomycin are both used in cancer therapy and are cytoxic. Silver lactate & acetic acid are acidic in nature.

**EXTREME CARE** should be used when handling either of these two items. The colloidal gold and colloidal silver solutions are potentially caustic and will temporarily discolor the skin. Most solutions contain 0.02% sodium azide as a preservative.

#### HEALTH HAZARD INFORMATION

None established. The toxicological properties of these products have
not been thoroughly investigated. Care should be taken when handling
any of these materials.
Any of these proteins may cause acute localized eye, skin, or mucous
membrane irritation. Some sensitive individuals may develop a chronic
attengic reaction with exposure.
Skind eye, and mucous membrane contact. Care should be taken to avoid
the formation of aerosols when handling any of these solutions.

#### PHYSICAL CHARACTERISTICS APPEARANCE: For colloidal g

For colloidal gold and its protein conjugates, light burgundy to purple liquid. 2nm - pale yellowish-brown liquid. All liquids are completely miscible in water and biological buffers.

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MSDS for Colloidal Gold Labeled Proteins, Enzymes, and Ligands Continued - page 2 of 2.

#### FIRE AND EXPLOSION HAZARDS

EXTINGUISHING MEDIA: SPECIAL FIRE FIGHTING PRECAUTIONS: NOTE:

# REACTIVITY DATA

STABILITY:

HAZARDOUS POLYMERIZATION: INCOMPATIBILITY:

#### SPILL / LEAK PROCEDURES

MATERIAL RELEASE / SPILL: Avoid of towel so to dry o deterger MASTE DISPOSAL: Incinera accorda Not considered to be a fire hazard. Water spray or CO<sub>2</sub>. None required. Most solutions contain 0.05% sodium azide as a preservative. Azide may react with lead and copper plumbing to form explosive metal azides. Flush with copious amounts of water when disposing material in the sink.

Stable. Decomposition products are not known to be hazardous. Will NOT occur. None known. (Lead and copper may react with sodium azide).

Avoid contact with liquid. Clean up spill with a paper towel soaked in household bleach. Do not allow solutions to dry on environmental surfaces. Wash affected area with detergent after the area has been treated with bleach. Incinerate, autoclave, or dispose of paper waste in accordance with all Local, State, and Federal regulations. Due to the small quantities of material involved these products are generally not considered to be environmental hazards. All of these proteins are fully biodegradable.

### **EMERGENCY FIRST AID PROCEDURES**

May be harmful if swallowed, inhaled, or allowed to absorb through the skin. Wash contacted area with water for 15 minutes. If inhaled remove to fresh air. Report exposure to the appropriate safety official. The gold and silver sols may be caustic. Consult a physician if irritation occurs, if there is any indication of an allergic response such as watering eyes, sneezing, or difficulty breathing, or if eye contact occurs.

#### SPECIAL HANDLING PRECAUTIONS

VENTILATION:	No special ventilation is required but it is recommended to
	handle these reagents in a fume hood when possible.
EYE PROTECTION:	Safety goggles or safety glasses with side shields are
	recommended.
RESPIRATORY PROTECTION:	Recommended as a safety precaution. An approved
	respirator may be required for those individuals already
	known to be sensitive to these materials.
PROTECTIVE GLOVES:	Required when handling any of these materials.

#### SPECIAL PRECAUTIONS

This material is for research and experimental application only. It is not intended for food, drug, household, agricultural, or cosmetic use. All materials should be handled only by technically qualified individuals experienced with working with potentially hazardous chemicals. The above information is correct to the best of our knowledge. The user should make independent decisions regarding completeness of the information, based on all sources available. EY Laboratories, Inc. shall not be held liable for any damage resulting from handling or contact with the above product.



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