# Immunogold Staining Kit #2 for EM

(Cat. No.: IGS-02)

## INTENDED USE

The IGS-02 is designed for the detection of primary antibodies directed against tissue and cell surface antigens through the use of Protein A or secondary antibody labeled Gold Colloidal Particles.

## SUMMARY AND PRINCIPLES OF PROCEDURES

Protein A or secondary antibody coated Gold Colloidal Particles have been used successfully for the detection of tissue and cell surface antigens. The Colloidal Gold appears as electron opaque spheres at the antigen site. This allows for localization of the binding site and a relative quantification of the number of binding sites present.

## STORAGE CONDITIONS

Colloidal Gold Particles should be stored at 5-8°C. **DO NOT FREEZE!** All other solutions and buffers may be stored at room temperature. Crystals may form if solutions are refrigerated. Redissolve by warming at room temperature.

## SAMPLE PROCESSING

After the sample has been mounted on a grid it is ready to be stained.

## RECOMMENDED PROCEDURE

1. Place the grids with the plastic thin sections in a droplet of reagent C for 5 minutes at Room temperature.
2. Transfer the grids onto a droplet (Approx. 15 microliters) of your antibody which has been appropriately diluted with Reagent E and Incubate for 1-2 hours at room temperature of 18-48 hours at 4° C.
3. Rinse 2 times for 2 minutes each time with a mild spray of Reagent C.
4. Incubate the sections on the grid with a mild spray of Reagent L (5nm GCP) or Reagent M (15nm GCP) for 1 hr at room temperature.
5. Rinse 2 times for 5 minutes each time with a mild spray of Reagent E and Finally with distilled water for 5 minutes. Dry the sections.
6. Counterstain with uranyl acetate and lead citrate. Dry the sections.
7. Review under the EM.



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(Outside CA only)

**Comments:** If too high background is observed dilute your primary antibody further with solution E, and/or incubate with primary antibody at 4°C.

## MATERIALS SUPPLIED

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REAGENT C: PBS, sodium azide, ovalbumin
REAGENT E: PBS, sodium azide, BSA, TRITON X-100, Tween 20
REAGENT L: Protein A – Gold Colloidal Particles (15nm)
REAGENT M: Protein A – Gold Colloidal Particles (5nm)

### MATERIAL NOT SUPPLIED

Tissue section mounted on a grid
Specific primary antibody
Uranyl acetate
Lead Citrate

## QUALITY CONTROL

It is recommended that the gold labeled reagents be checked for activity using the enclosed Dot Blot prior to use. GCP solution should be red in color. **DO NOT USE** if it is blue or if precipitate is present.

## LIMITATIONS OF PROCEDURE

1) Solutions containing detergents such as triton X-100 and TWEEN 20 may reduce background staining by removing non-specifically absorbed proteins, however, this solution may also remove some of the protein to be detected if it is overused.
2) Proper dilutions of the primary antibody and the gold labeled reagent must be determined by the researcher for the individual experiment.

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## TROUBLE SHOOTING GUIDE

| PROBLEM | CAUSE | SOLUTION |
|---|---|---|
| Weak or No Staining | 1. Inappropriate fixation and embedding.<br>2. Low antigen or substrate concentration.<br>3. Inactive reagents due to long storage. | 1. a) Use Other Fixatives and shorter fixation times.<br>b) Omit embedding and use frozen sections.<br>c) Prepare unfixed cryostate sections.<br>2. a) Use Silver Enhancement technique.<br>b) Prolong antibody incubation time at Room Temperature or 4°C.<br>c) Use further bridging steps with unlabeled reagents for amplification.<br>d) Use fresh reagents.<br>3. Replace with fresh reagent. |
| High Background | 1. Primary and/or secondary antibody, Gold Complex to concentrated.<br>2. Insufficient washing.<br>3. Insufficient blocking.<br>4. Insufficient blocking of aldehyde groups. | 1. a) Decrease concentration of the respective reagents.<br>b) Increase detergent concentration.<br>c) Shorten the incubation times.<br>2. Perform multiple washings and Prolong washing time.<br>3. Prolong blocking time with Reagent E.<br>4. Treat Tissue or Tissue sections with 50mM $NH_4CL$ in buffer for 30-60 minutes. |
| Unexpected staining pattern | 1. Multiple causes | a) Perform adequate cytochemical control reactions.<br>b) Proper control of antibody quality, exclusion of crossreactivity<br>c) Use other cytochemical technique to prove or disprove the findings. |



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## REFERENCES

1) Horisberger, M., In "Scanning Electron Microscopy" (O'Jahari, Ed.), Vol. II., 9, 1981. SEM Inc., O'Hare.
2) Roth, J., In "Techniques in Immunocytochemistry" (Eds. G.R. Bullock and P.Petrusz), VOL.II, 217, 1983; Academic Press, London, New York.
3) Geoghegan, W.D., Scillian, J.J., and Ackerman, G.A., Immunol. Commun. 7,1,1978.
4) Roth, J., In "Advances in Immunohistochemistry" (Ed. R.A. DeLellis), Page 63, Masson Publ. USA, Inc. New York, 1984.
5) Ackerman, G.A., and Wolken, K.W., J. Histochem. Cytochem. 29, 1137, 1981.

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# MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006
Revision 4


## PRODUCT IDENTIFICATION

Name: Colloidal gold and colloidal silver labeled proteins, enzymes, and ligands.

Catalog Number(s): G-2 to G-40, XGP-2, GP-01 to GP-8006, GAP-01, FGP-01, HGP-01, RGP-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, GE-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, IGS-01, IGS-02, LGS-01.

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.

## EMERGENCY INFORMATION

EY Laboratories, Inc.
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**EMERGENCY PHONE:**
**650-342-3296**

## HAZARDOUS COMPONENTS

Specific protein or ligand 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.

**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

EXPOSURE LIMITS: None established. The toxicological properties of these products have not been thoroughly investigated. Care should be taken when handling any of these materials.

EFFECTS OF OVEREXPOSURE: Any of these proteins may cause acute localized eye, skin, or mucous membrane irritation. Some sensitive individuals may develop a chronic allergic reaction with exposure.

ROUTES OF EXPOSURE: Skin, eye, and mucous membrane contact. Care should be taken to avoid the formation of aerosols when handling any of these solutions.

## PHYSICAL CHARACTERISTICS

APPEARANCE: Light burgundy to purple liquid. 2nm - pale yellowish-brown liquid.
SOLUBILITY: All liquids are completely miscible in water and biological buffers.



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## FIRE AND EXPLOSION HAZARDS

Not considered to be a fire hazard.
EXTINGUISHING MEDIA: Water spray or $CO_2$.
SPECIAL FIRE FIGHTING PRECAUTIONS: None required.
NOTE: 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.

## REACTIVITY DATA

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

## SPILL / LEAK PROCEDURES

MATERIAL RELEASE / SPILL: 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.

WASTE DISPOSAL: 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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