Immunogold Staining Kit #1 for LM

(Cat. No.: IGS-01)

INTENDED USE

The IGS-01 contains reagents to detect antibodies directed against cell and tissue surface antigens.

SUMMARY AND PRINCIPLES OF PROCEDURES

In the past, surface antigens labeled with polyclonal or monoclonal primary antibodies have been visualized using fluorescent or enzyme labeled secondary antibodies. Both techniques have limitations, fluorescent dyes tend to photobleach and enzyme labels require an enzymatic reaction after incubation in order to visualize the labeling. In both cases, it is necessary to perform several experiments in order to determine the proper incubation times that will give the most labeling with the least background. Through the use of Protein A-Gold or Secondary Antibody-Gold it is possible to monitor the degree of labeling during incubation. The use of Silver Enhancement techniques make it possible to detect two different antigens on the same section. The immunogold staining technique can be as sensitive as other labeling procedures and results in a permanent, non-photosensitive stain.

STORAGE CONDITIONS

Colloidal Gold Particles should be stored at 5-8°C. **DO NOT FREEZE!** Buffer solution should be stored at room temperature. Crystals may form if solution is refrigerated. Redissolve by warming at room temperature.

SAMPLE PROCESSING

1. Incubate cell suspension, rehydrated paraffin, frozen or semi-thin section with your specific antibody (polyclonal or monoclonal antibody appropriately diluted with Reagent E) for 2 hours at room temperature. The proper concentration of the primary antibody must be determined by the researcher.
2. **Do not** use Reagent E to dilute antibody when using cell suspensions since it contains detergents with will cause cell lysis. Instead, use the buffer in which the cells are already suspended and add 1% BSA or 1% ovalbumin.

NOTE: When Protein A-GCP is used it is best to use a primary antibody that is of rabbit origin **or** incubate with a rabbit secondary antibody directed against the primary antibody. This is necessary due to the varying reactivity of Protein A with immunoglobins from different species. (Ref. 1) Where a secondary antibody is not available, EY Labs can substitute Goat Anti-Mouse IgG, Goat Anti-Human IgG, Goat Anti-Rabbit IgG, or Rabbit Anti-Goat IgG - Gold Colloidal Particles for the Protein A Gold in this kit.

EY LABORATORIES, INC.
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(Outside CA only)

RECOMMENDED PROCEDURE

A. Tissue sections
1. Dilute secondary antibody (if available) to 50µg/ml using Reagent E. Incubate with section for 45 minutes to 1hr. at room temperature.
2. Rinse section twice for 5 minutes each time in Reagent E. Dry area around section but do not let sample dry.
3. Reagent M is prepared by diluting with Reagent E to give a final OD 525 of 1.7. This gives the optimum concentration for light microscopy. The same conditions apply when using Secondary Antibody-Gold Particles.
4. Place a drop or two of Reagent M to cover section.
5. The buffer used for rinsing can act as a diffusion boundary, to insure that the Gold Reagent is in contact with the section, **gently** pipette solution up and down to mix. Incubate for 30 minutes to 1 hr.

NOTE: Use a moisture chamber for incubation. Longer incubations may be necessary depending on the researcher's application. It is possible to monitor the progress of the labeling using phase contrast or epi-polarization microscopy. Do not confuse the overall pinkish stain of the gold solution with the pink stain outlining the structures being studied.

6. Rinse twice with Reagent E for 5 minutes each time followed by distilled water. Counterstaining of the sections, if needed, may be done using methyl green.
7. Dehydrate the sections rapidly through 70%, 90%, and 100% (twice) ethanol and finally twice in xylene.
8. Use Silver Enhancement technique to intensify latent staining if necessary.
9. Mount in Canada Balsam.

B. Cell Suspension
1. Follow the procedures as outline in A for Tissue Sections but be careful to maintain isomotic conditions. **Do not** use distilled water or Reagent E since both will cause cell lysis. Use the same buffer in which the cells are suspended for all washing and dilutions steps. Include either 1% BSA or 1% ovalbumin in the buffer.

C. Double labeling
1. Folllow steps 1-5 as above using Protein A-Gold and first primary antibody. Rinse with Reagent E as described and distilled water three times.
2. Follow Silver Enhancement directions. **DO NOT MOUNT.**
3. Rinse with Reagent E and repeat labeling procedure using second primary antibody. Omit Silver Enhancement step.
4. Proceed with dehydration and mounting.

NOTE: The first primary antibody will be stained black with the silver and the second primary antibody will appear pink-red.

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MATERIALS REQUIRED

MATERIAL SUPPLIED

REAGENT E: PBS, Tween 20, Triton X-100, BSA NaN_3
REAGENT M: Protein A – Gold Colloidal Particles (15nm) (May be substituted with GxR IgG, GxH IgG, GxM IgG, RxG IgG-GCP)

MATERIAL NOT SUPPLIED

Primary monoclonal or polyclonal antibody
Rabbit secondary antibody
Moisture Chamber
Embedding Media
Methyl Green
Ethanol, Xylene
Canada Balsam

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

Protein A does not react well with IgG from all species. Please refer to Reference 1 for more information.

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TROUBLE SHOOTING GUIDE

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

REFERENCES

A) Lindmark, R., et al. J. Immuno, Meth. 1983, 62, 1

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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:
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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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