

Lectin Gold Staining Kit

General Information

(Cat. No.: LGS-01)

INTENDED USE

This kit is designed for light and electron microscopic study of Glycoproteins on cell surfaces and tissue sections.

Summary and Principles of Procedures

The LGS-01 Kit is used to demonstrate the presence of specific carbohydrates in Glycoproteins on tissue sections through the use of Lectins combined with the Colloidal Gold marker system. For light microscopy the presence of Lectin binding sites is revealed by the appearance of a reddish color. Latent Gold staining can be detected through the use of the Silver Enhancement Technique. This deposits metallic silver on the surface of the Gold Colloidal Particles (GCP), which changes the red color to a brownish-black. For electron microscopy Lectin binding sites are visualized by the appearance of electron dense spheres, the Colloidal Gold Complex produces A permanent, non-bleaching stain.

Storage Conditions

Gold Colloidal Particles should be stored at 5-8°C. **DO NOT FREEZE!** Lyophilized Lectins may be stored frozen in powdered form or aliquot and stored frozen after reconstitution. 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

- A. Frozen sections:** Rehydrate using reagent G (for LFA or LPA and Con A labeling only) or reagent A for all other lectins and glycoproteins.
- B. Paraffin sections:** For removal of the paraffin place sections in xylene (2 times, 5 minutes each time) (Rehydrate each time) Followed by 90%, 70%, 50% and 30% ethanol (5 minutes each time). Place sections in reagent G (for LFA or LPA and Con A labeling only) or reagent A for all other lectins and glycoproteins for 5 minutes.
- C. Semi-thin plastic sections:** Remove the plastic (Epon or Araldite) by exposing the section to a solution consisting of 2gm KOH, 5ml propylene oxide and 10ml methanol for 3-5 minutes. Rinse with an equal volume mixture of methanol: Reagent G. Finally, rinse with reagent G (for LFA or LPA and Con A labeling only) or reagent A for all other lectins and glycoproteins.

Recommended Procedure

Note: Please read entire procedure before beginning experiment. The direct staining procedure is used for PNA, DBA and UEA-I GCP. The indirect staining procedure is used for Con A, LFA or LPA and WGA lectins utilizing a specific glycoprotein-GCP. Con A and LFA or LPA use reagent G for all dilutions and washing steps. Reagent A may be used for all other lectin and glycoprotein-GCP.

- A. Direct staining procedure-Light Microscopy**
1. Incubate section in Lectin-GCP solution in a moist chamber for 3-90 minutes at room temperature. Dilute lectin-GCP using reagent A. For light microscopy the optimum staining occurs using A gold solution with an OD520=1.7. More dilute material may be used but require longer incubation times. **DO NOT DISCARD GOLD SOLUTION AFTER USE.** It may be re-used until there is a noticeable decrease in staining.
 2. Rinse sections with reagent A (2 times, 5 minutes each time) and finally with distilled water.
 3. Use Silver Enhancement Kit to increase signal of latent staining if the red color if the GCP does not appear distinctly when viewed under the microscope. Be sure to rinse extensively with distilled water prior to using the silver reagents to remove any chloride ions present.
 4. Dehydrate the sections rapidly through 70%, 90%, 100% ethanol and twice with xylene.
 5. Mount section.
- B. Indirect staining procedure-Light Microscopy**
1. Dilute Con A and LFA or LPA with reagent G. Dilute WGA with reagent A.
 2. Incubate rehydrated sections in lectin solution (diluted to 200µg/ml) for 30-90 minutes at room temperature. **DO NOT DISCARD LECTIN SOLUTION AFTER USE.** It may be re-used until there is a noticeable decrease in staining.
 3. Rinse with reagent A or G (2 times, 5 minutes each time).
 4. Dilute appropriate glycoprotein-GCP with either reagent A or reagent G. For light microscopy the optimum staining occurs using A Gold solution with an OD520 of 1.7 More dilute material may be used but require longer incubation times. **DO NOT DISCARD GOLD SOLUTION AFTER USE.** It may be re-used until there is a noticeable decrease in staining.
 5. Rinse sections with reagent A or G (2 times, 5 minutes each time) and finally with distilled water.
 6. Use Silver Enhancement Kit to increase signal of latent staining if the red color of the GCP does not appear distinctly when viewed under the microscope. Be sure to rinse extensively with distilled water prior to using the silver reagent to remove any chloride ions present.
 7. Dehydrate the section rapidly through 70%, 90%, 100% ethanol and twice with xylene.
 8. Mount section.

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Special Instruction for Electron Microscopy

The Lectin Gold staining kit may be used for pre- or postembedded sections. For postembedded staining the section must be etched and mounted on the grid prior to staining with the gold reagents.

- C. Direct postembedding staining on ultrathin sections-electron microscopy.
- Place grids sample side down on a droplet of reagent A for 5 minutes.
 - Place grids sample side down on a droplet of lectin-GCP diluted with reagent A. The dilution will vary from experiment to experiment but we recommend starting with a 1:30-1:40 dilution, incubate 30-60 minutes at room temperature.
 - Rinse gently with reagent A (2 times, 5 minutes each time) and finally with distilled water.
 - Counterstain lowicryl with uranyl acetate and lead citrate. Counterstain frozen sections according to REF 1.
- D. Indirect Postembedding staining on ultrathin sections-electron microscopy.
- Place grids sample side down on a droplet of reagent A or G for 5 minutes at room temperature.
 - Transfer grids to A droplet of diluted Lectin solution (200µg/ml) and incubate for 1 hour at room temperature.
 - Rinse gently with reagent A or G (2 times, 5 minutes each time).
 - Place grids sample side down on a droplet of the appropriate glycoprotein-GCP which has been diluted with reagent A or G. The dilution will vary from experiment to experiment but we recommend starting with A 1:30-1:40 dilution. Incubate 3-60 minutes at room temperature.
 - Rinse gently with reagent A or G (2 times, 5 minutes each time) and finally with distilled water.
 - Counterstain lowicryl with uranyl acetate and lead citrate, Counterstain frozen sections according to REF.1
- E. Cell Surface Staining
- Similar reagent dilutions and incubation times may be used as described above to study cell surfaces redistribution phenomena and endocytosis. Take care to maintain isosmotic conditions at all times. **DO NOT RINSE WITH DISTILLED WATER.**

MATERIAL REQUIRED

- A. Materials Supplied
- Reagent A - Concentrated phosphate buffered saline pH 7.45
 - Reagent G - Concentrated Tris buffered saline pH 7.2
 - Glycoprotein-GCP
 - HRP-GCP (for use with Con A)
 - Ovomucoid GCP (for use with WGA)
 - Fetuin GCP (for use with LFA or LPA)

- Lectin
 - PNA-GCP
 - DBA_GCP
 - UEA-I-GCP
 - Con A
 - WGA
 - LFA or LPA
- Inhibitory Carbohydrate
 - Lactose
 - N-Acetyl-D-Galactosamine
 - L-Fucose
 - Alpha-Methylmannopyranoside
 - N-Acetyl-D-Glucosamine
 - Sialic Acid

B. Materials Not Supplied

- Ethanol, xylene, Koh, Propylene oxide, methanol
- Counterstaining and mounting reagents
- Moist chamber

LIMITATIONS OF PROCEDURES

The dilutions given for the Lectin-GCP and Glycoprotein-GCP reagents may need to be adjusted for individual experiments. Any proteins used as blocking agents must be non-reactive and must not contain any carbohydrate components which may interfere with Lectin Specificity.

TROUBLE SHOOTING GUIDE FOR LECTIN GOLD STAINING KIT

Problem	Cause	Solution
Weak or Staining	1. Inappropriate and fixation and embedding	A. Use other fixatives and Shorter fixation times B. Omit Embedding and use frozen sections. C. Prepare unfixed cryostat section.
	2. Low Glycoprotein Concentration.	A. Prolong incubation time with Lectin and/or glycoprotein-GCP B. Use Silver Enhancement Kit.
	3. Inactive reagents due to long storage.	A. Use fresh reagents
High Background	1. Lectin and/or Glycoprotein-GCP	A. Decrease concentration of respective reagents. B. Use shorter incubation times.
	2. Insufficient washing.	A. Perform multiple washings. B. Increase reactions.
Unexpected Staining	1. Multiple causes.	A. Perform adequate cytochemical control reactions.
		B. Use other cytochemical technique to prove or disprove the findings.

REFERENCES

- Tokuyasu, et. Al phas,(1981), 78.7619.
- Maxwell, J. Microscopy (1978), 112, 253.
- Roth, J., J. Histochem Cytochem. (1982) 30, 691.
- Roth, J., J. Histochem Cytochem. (1983) 31, 547.

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MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006

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

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

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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 cytotoxic.

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.

FIRE AND EXPLOSION HAZARDS

EXTINGUISHING MEDIA:

SPECIAL FIRE FIGHTING PRECAUTIONS:

NOTE:

Not considered to be a fire hazard.

Water spray or CO₂.

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.

REACTIVITY DATA

STABILITY:

HAZARDOUS POLYMERIZATION:

INCOMPATIBILITY:

Stable. Decomposition products are not known to be hazardous.

Will NOT occur.

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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MSDS for Crude and Purified Proteins and Enzymes Continued - page 2 of 2.

PRODUCT IDENTIFICATION

Name: Crude and purified protein and enzymes.
Catalog Number (s): P-01, 2402, 2404, EC-32118, EC-32118S, E-34424, EC-34424, BA-000, BA-002, NP-01 to NP-05, B-1201 to B-4601, L-1102 to L-9000, AT-2100 to AT-2701, AF-001 to AF-2354, AL-1104 to AL-4701, 13-600 to 13-607, DM1011P to DM1064P, LGS-01, SAQD-01, SAQD-02.
Formula: Complex polypeptides.
Synonyms: Protein A, Horseradish Peroxidase, Laminin (mouse), Neuraminidase, Bromelain, Avidin (egg white), Glycosylated Bovine Serum Albumin, Lectins, Secondary and Monoclonal Antibodies, other Antisera.

EMERGENCY INFORMATION

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HAZARDOUS COMPONENTS

Specific protein (s) as listed on the vial label. Solutions are at a concentration generally greater than 0.5mg protein/ml. Powders are generally greater than 95% specific protein unless otherwise indicated on the vial label or product information sheet. 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.

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: Inhalation of powders and skin contact with liquids are the primary routes of exposure. Care should be taken to avoid the formation of aerosols when handling any of the solutions.

PHYSICAL CHARACTERISTICS

APPEARANCE: Powders may be white to amber brown in color. Solutions may be translucent to a clear brown
SOLUBILITY: Powders are completely soluble in many biological buffers. Some are soluble in water. All liquids are completely miscible in water and biological buffers.

FIRE AND EXPLOSION HAZARDS

EXTINGUISHING MEDIA: Not considered to be a fire hazard.
SPECIAL FIRE FIGHTING PRECAUTIONS: Water spray or CO₂.
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 powder or 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. Consult a physician if irritation occurs or if there is any indication of an allergic response such as watering eyes, sneezing, or difficulty breathing

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: Not required under most circumstances but recommended as a safety precaution.
RESPIRATORY PROTECTION: Recommended as a safety precaution, specifically when working with powders. 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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MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006

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PRODUCT IDENTIFICATION

Name: Monosaccharides, Disaccharides, and Polysaccharides.
Catalog Number (s): C-6000 to C-8006, LGS-01, SAQD-01, SAQD-02.
CAS Numbers: 14215-68-0, 10036-64-3, 6696-41-9, 59-23-4, 6363-53-7, 3458-28-4, 585-99-9, 492-62-6, 131-48-6, 617-04-9, 5989-81-1, 70431-34-4, 11040-27-0, 32181-59-2.
Synonyms: N-Acetyl-D-Galactosamine, N-Acetyl-D-Glucosamine, α -L-Fucose, D-Galactose/Maltose, D-Mannose, Melibiose, D-Glucose, N-Acetylneuraminic Acid (sialic acid and oligomers of sialic acid), Methyl α -D-Mannopyranoside, α -Lactose, Colominic Acid, N-Acetylneuraminy Lactose (sialyllactose), Chitobiose (diacetyl chitobiose), chitotriose (triacetyl chitotriose), N-acetyllactosamine, Methyl β -D-Mannopyranoside Isopropylate.

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HAZARDOUS COMPONENTS

These carbohydrates are not considered to be hazardous but they may cause irritation or an allergic response in sensitive individuals.

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: No effects of overexposure have been documented. The carbohydrates may cause irritation or an allergic reaction
ROUTES OF EXPOSURE: Ingestion, inhalation, or contact with skin. Contact with the eyes may also present a hazard.

PHYSICAL CHARACTERISTICS

APPEARANCE: White to tan powder.
SOLUBILITY: Soluble in water.

FIRE AND EXPLOSION HAZARDS

EXTINGUISHING MEDIA: Not considered to be a fire hazard.
SPECIAL FIRE FIGHTING PRECAUTIONS: Water spray or CO₂.
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.

MSDS for Monosaccharides, Disaccharide and Polysaccharides Continued - page 2 of 2.

REACTIVITY DATA

STABILITY: Stable. Decomposition products are not known to be hazardous.
HAZARDOUS POLYMERIZATION: Will NOT occur.
INCOMPATIBILITY: None known.

SPILL / LEAK PROCEDURES

MATERIAL RELEASE / SPILL: Clean up spill with soap and water.
WASTE DISPOSAL: Incinerate, autoclave, or dispose of paper waste in accordance with all Local, State, and Federal regulations. These materials are fully biodegradable.

EMERGENCY FIRST AID PROCEDURES

May be harmful if swallowed, injected, or allowed to contact the eyes. Wash contacted area with water for 15 minutes. If inhaled remove to fresh air. Report exposure to the appropriate safety official. Consult a physician if irritation occurs or if there is any indication of an allergic response, such as watering eyes, sneezing, or difficulty breathing. Any eye contact should be reported to a physician immediately.

SPECIAL HANDLING PRECAUTIONS

VENTILATION: No special ventilation is required.
EYE PROTECTION: Recommended but not required for most applications.
RESPIRATORY PROTECTION: Recommended to prevent inhalation of powders. An approved respirator may be required for those individuals already known to be sensitive to these materials.
PROTECTIVE GLOVES: Recommended but not required for most applications. Required for those individuals known to be sensitive to 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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