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.

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(Outside CA only)

Recommended Procedure

Note: Please read entire procedure bef inning experiment. The direct staining procedure is used for PNA, DBA and UE . The indirect staining procedure is used for Con A, LFA or LPA and WGA lectin ng a specific glycoprotein-GCP. Con A and LFA or LPA use reagent G for all c and washing steps. Reagent A may be used for all other lectin and glycoprotein-C

A. Direct staining procedure-Light Mic

1. Incubate section in Lectin-GC ion in a moist chamber for 3-90 minutes at room temperature. Dilute le CP using reagent A. For light microscopy the optimum staining occurs u 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 decease 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 ultranthin sections-electron microscopy.

1. Place grids sample side down on a droplet of reagent A for 5 minutes.
2. 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.
3. Rinse gently with reagent A (2 times, 5 minutes each time) and finally with distilled water.
4. Counterstain lowicryl with uranyl acetate and lead citrate. Counterstain frozen sections according to REF 1.

D. Indirect Postembedding staining on ultrathin sections-electron microscopy.

1. Place grids sample side down on a droplet of reagent A or G for 5 minutes at room temperature.
2. Transfer grids to A droplet of diluted Lectin solution (200µg/ml) and incubate for 1 hour at room temperature.
3. Rinse gently with reagent A or G (2 times, 5 minutes each time).
4. 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.
5. Rinse gently with reagent A or G (2 times, 5 minutes each time) and finally with distilled water.
6. Counterstain lowicryl with uranyl acetate and lead citrate, Counterstain frozen sections according to REF.1

E. Cell Surface Staining

1. Similar reagent dilutions and incubation times may be sued 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 REQUIRD

A. Materials Supplied

1. Reagent A - Concentrated phosphate buffered saline pH 7.45
2. Reagent G - Concentrated Tris buffered saline pH 7.2
3. Glycoprotein-GCP
   HRP GCP (for use with Con A)
   Ovomucoid GCP (for use with WGA)
   Fetuin GCP (for use with LFA or LPA)
4. Lectin
   PNA-GCP
   DBA_GCP
   UEA-I-GCP
   Con A
   WGA
   LFA or LPA

   hibitory Carbohydrate
   actose
   l-Acetyl-D-Galactosamine
   -Fucose
   lpha-Methylmannopyranoside
   l-Acetyl-D-Glucosamine
   ialic Acid

B. Materials Not Supplied

1. Ethanol, xylene, Koh, Propyle e, methanol
2. Counterstaining and mouting ;
3. 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

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4. Roth, J., J. Histochem Cytochem. (1983) 31, 547.

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