SIALIC ACID DETECTION KIT

Catalog Number: SAD-01

INTENDED USE

The Sialic Acid Detection Kit is designed for detection of sialic acid residues on tissue sections and cell surfaces.

SUMMARY AND PRINCIPLES OF PROCEDURES

Glycoproteins containing sialic acid have been localized in the past using fluorescent and enzyme labeled lectins such as Limulus polyphemus agglutinin (LPA) and Limax flavus agglutinin (LFA). The Sialic Acid Detection Kit uses an indirect staining method in order to localize receptors for light and electron microscopic studies.[1] The kit contains purified LFA which binds to sialic acid residues of the tissue or cell being studied. A glycoprotein labeled gold colloidal particle, Fetuin-GCP, is then used to localize bound LFA. This indirect staining method is employed in order to avoid problems of steric hindrance which may occur on tissue and cell surfaces. In cases where staining pattern is weak it is possible to amplify stain by using the Silver Enhancement Kit provided.

STORAGE CONDITIONS

Store lyophilized LFA refrigerated at 5-8°C. Store liquid frozen in aliquots. Avoid freeze-thaw cycles. Clarify by centrifugation. 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

FROZEN SECTIONS

Rehydrate sections by placing sections in 0.01M Phosphate Buffered Saline (PBS), pH 7.2.

PARAFFIN SECTIONS

For removal of paraffin, place slides with sections in xylene (2 times, 5 minutes each time). Rehydrate sections through 100% ethanol (2 times, 5 minutes each time) followed by 90%, 70%, 50% and 30% ethanol (5 minutes each time). Place slides in REAGENT G for 5 minutes.

SEMITHIN PLASTIC SECTIONS

Remove plastic (Epon or Araldite) by exposing section to a solution consisting of 2 g KOH, 5ml propylene oxide and 10ml methanol for 3-5 minutes.[2] Rinse with methanol: water (v:v). Finally, rinse with REAGENT G alone.

RECOMMENDED PROCEDURE

FROZEN, PARAFFIN, OR SEMI-THIN SECTIONS

1. Dilute LFA (to 100µg/ml) with REAGENT G.
2. Incubate rehydrated sections in LFA solution for 1 hour in a moist chamber at room temperature. DO NOT DISCARD solution after use. It may be re-used until there is a noticeable decrease in staining.
3. Rinse with REAGENT G (2 times, 5 minutes each time).
4. Dilute Fetuin-GCP (15nm) with REAGENT G to an OD (525nm) = 1.0.
5. Incubate section using Fetuin-GCP for 30-90 minutes in a moist chamber at room temperature. DO NOT DISCARD gold solution after use. It may be re-used until there is a noticeable decrease in staining.
6. Rinse sections with REAGENT G (2 times, 5 minutes each time) and finally with distilled water.
7. Use Silver Enhancement Kit to increase signal of latent staining if red color of the GCP does not appear distinctly when viewed under microscope. Rinse extensively with distilled water prior to using the silver reagent to remove any chloride ions present.
8. Dehydrate sections rapidly through 70%, 90%, 100% ethanol and twice with xylene.
9. Mount section.

POSTEMBEDDING STAINING ON ULTRATHIN SECTIONS

(Low temperature embedding with Lowicryl K4M or ultrathin frozen sections)[3]

1. Place grids, sample side down, onto a droplet of REAGENT G for 5 minutes at room temperature.
2. Dilute LFA to 100µg/ml with REAGENT G.
3. Transfer grids onto a droplet of LFA solution and incubate for 1 hour at room temperature in a moist chamber.
4. Rinse gently with REAGENT G (2 times, 5 minutes each time).
5. Dilute Fetuin-GCP (5nm) with REAGENT G to an OD (525nm) = 0.35.
6. Place grids onto a droplet of the diluted Fetuin-GCP and incubate for 30-90 minutes in a moist chamber at room temperature. DO NOT DISCARD gold solution after use. It may be re-used until there is a noticeable decrease in staining.
7. Rinse gently with REAGENT G (2 times, 5 minutes each time) and finally with distilled water.
8. Counterstain Lowicryl with uranyl acetate and lead citrate. Counterstain frozen sections according to Reference 3.

CELL SURFACE STAINING

1. 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.

CONTROLS FOR SPECIFICITY

Specificity of lectin binding can be tested by addition of appropriate inhibitory carbohydrate to the lectin 30 minutes before use. The proper concentration is dependent upon the inhibitory carbohydrate being used. EY Laboratories, Inc. recommends 10mM Sialic Acid.

MATERIALS SUPPLIED

REAGENT G	:	Concentrated Tris Buffered Saline, pH 7.2
Lectin	:	Purified LFA
Glycoprotein GCP	:	Fetuin - GCP (5nm and 15nm)
SET-10	:	Silver Enhancement Kit

MATERIALS NEEDED BUT NOT SUPPLIED

Xylene, ethanol, KOH, propylene oxide, methanol
Embedding reagents
Conterstaining reagents
Moist Chamber

LIMITATIONS OF PROCEDURE

The dilutions given for the Fetuin - GCP in the recommended procedure 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 binding.

TROUBLE SHOOTING GUIDE

Problem	Cause	Solution
Weak or staining	1. Inappropriate fixation and embedding.	a. Use other fixatives and shorter fixation times. b. Omit embedding and use frozen sections. c. Prepare unfixed cryostat sections.
	2. Low glycoprotein concentration.	a. Prolong incubation time with LFA and/or Fetuin-GCP. b. Use Silver Enhancement Kit.
	3. Inactive reagents due to long storage.	a. Use fresh reagents.
High Background	1. LFA and/or Fetuin-GCP concentration too high.	a. Decrease concentration of respective reagents. b. Decrease incubation times.
	2. Insufficient washing.	a. Perform multiple washings. b. Increase washing times.
Unexpected staining	1. Multiple causes.	a. Perform adequate cytochemical control reactions. b. Use other cytochemical techniques to prove or disprove the findings.

REFERENCES

1. Roth, et al. (1984), J. Histochem. Cytochem. **32**, 1167-1176.
2. Maxwell, J. (1978), Microscopy. **112**, 253-255.
3. Tokuyasu (1983), J. Histochem. Cytochem. **31**, 164-167.

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MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006
Revision 5

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, SAD-01.

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

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

EMERGENCY PHONE: 650-342-3296

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

Not considered to be a fire hazard.

EXTINGUISHING MEDIA: Water spray or CO_2.

SPECIAL FIRE FIGHTING PRECAUTIONS: None required.

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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
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, SET-10, SAD-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, silver lactate, acetic acid.

EMERGENCY INFORMATION

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EMERGENCY PHONE:
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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

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:	For colloidal gold and its protein conjugates, 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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