Lectin Individual Gel Kit
(Cat. No.: AK-5901-2)

Introduction

The Lectin Gel Kit (AK-series) contains a lectin column, required carbohydrates, and recommended buffers necessary to run 5-10 experiments per lectin gel. Each lectin is coupled to agarose beads at a protein concentration that will permit release of most glycoproteins or glycopeptides with the carbohydrate solutions provided in the kit. Proteins that are heavily glycosylated may require higher carbohydrate concentrations for efficient elution. In such cases, additional carbohydrate may be added directly to the stock carbohydrate solutions provided in the kit.

The individual lectin columns are reusable and require a simple regeneration step. Many experiments may be run on the same lectin gel, limited only by the viscosity of the sample applied and the flow rate of the column. The gentle elution conditions permit retention of the biological activity of the glycoprotein. When stored properly, the immobilized lectin may be kept for several years with little loss in activity.

Kit Composition

Immobilized Lectins
Erythrina cristagalli (ECA), 2ml

Carbohydrate Solutions and Buffers:
0.1M Lactose in Buffer

Buffers

0.05M Tris - 0.15M NaCl-0.01M $CaCl_2$, pH 7.5 - 8.0

Immobilized Lectin Concentration

ECA 4-5 mg purified ECA per 1 ml settled beads

Lectin Specificity

ECA Galactose β(1,4) N-Acetylglycosamine

Specific Applications

In the past twenty years many applications for immobilized lectins have been published. These applications may be classified into two major categories: The characterization of oligosaccharides and the purification of glycoproteins or oligosaccharides. The characterization of oligosaccharides is discussed in the work of Prof. Toshiaki Osawa (Biotechs, Winter 1981, published by EY Laboratories, Inc., available upon request). Additional information is also found in the work of Prof. Richard D. Cummings (Methods in Enzymology, 1987 vol. 138, p.232). Selected applications describing the use of immobilized lectins in the isolation and purification of glycoproteins and oligosaccharides, including references, follow.

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Tel: 650-342-3296
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Orders: 1-800-821-0044
(Outside CA only)

Applications

1. Separation of subpopulations of mammilian β-andrenergic receptors.
2. Changing pattern in glycan branching and sialyation of Thy-1 antigen during normal differentiation of mouse lymphocytes.
3. Purification of insulin receptors.
4. Studies of the carbohydrate moiety of epidermal growth factor, low density lipoprotein receptors, and the murine major histocompatibility antigen.
5. Carbohydrate analysis of band 3 from adult human erythrocytes.
6. Carbohydrate analysis of human amniotic fluid fibronectin.
7. Isolation of viral glycoproteins.
8. Separation of complex oligosaccharide using different chromatographic techniques.
9. Isolation of glycocalicin from human platelet membranes.
10. Purification of membrane glycoproteins.
11. Detection of terminal, non-reducing α-galactosyl residues of glycoproteins and oligosaccharides.
12. Separation of immature from thymocytes by selective agglutination or affinity chromatography.
13. Separation of germ cells from somatic cells in mouse testis.
14. Study of the increased branching pattern of tumor glycoprotein in patients with pituitary tumors.

Procedures For Use

General Procedures

All lectin gels may be run either in the cold room or at room temperature. Elevated temperatures (above 25°C) should be avoided. Each gel is ready to use, but it is recommended that some buffer be added to the top of each column before applying sample. The flow rate of the column will vary depending on the viscosity of the sample and the operating temperature. A flow rate of 20ml/hour is recommended. Some detergents may affect specific lectin binding (Lotan, R., et al. 1977). The individual columns fit into a standard 16mm diameter test tube. The rack supplied with the kit may be used in the event that only a smaller tube is available.

The recommended buffer for Con A and LcH is Tris Buffered Saline (TBS). The recommend buffer for RCA-I, WGA, UEA-I, and PNA is Phosphate Buffered Saline (PBS). The specific carbohydrates for these lectins are supplied in the appropriate buffers. Pretreatment of the sample to remove insoluble material is required to maintain the flow of the column. The sample should also be suspended in, or dialyzed against the appropriate buffer before applying to the lectin column.

Specific Procedures

1. Apply 250-500µl of the recommended buffer to the surface of the lectin gel. Apply sample when the column goes dry. The amount of glycoprotein bound will depend on the degree of glycosylation and the molecular weight of the protein.
2. Wash any unbound material from the column using the appropriate buffer. Add 200-300µl of buffer at a time. Collect fractions of less than 0.5ml/tube.
3. Monitor the eluent by measuring the OD 280nm or by assaying for specific components in the sample. DO NOT OVERWASH!! Lectin affinity is dependent on the equilibrium between the lectin/carbohydrate complex and the free lectin and carbohydrate. Extensive washing of the lectin column will affect this equilibrium and permit a glycoprotein to elute from the column in the absence of the specific carbohydrate.

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4. Elute with specific carbohydrate as indicated below for each lectin. Apply 200-300µl of solution at a time (approximately 7-8 drops). Collect small fractions as described above. In general, most glycoproteins will elute using 2-3 gel volumes of the carbohydrate solution.

   ECA 0.1M Lactose in Buffer.

Trouble Shooting

Some glycoproteins may remain tightly bound to the matrix when eluted with the carbohydrate solution provided. This is usually an indication of a highly glycosylated protein. The addition of free carbohydrate to the solution supplied in the kit, in order to increase the molarity, may be sufficient to elute tightly bound glycoproteins.

Solutions may be increased to a final concentration of 0.5M with the exception of lactose. Lactose does not dissolve readily at ambient temperatures at a concentration greater than 0.25M. Repeat the elution procedure with the concentrated carbohydrate solution as described in the above procedures.

Column Regeneration

Regeneration serves two purposes: to remove non-specifically bound material and to remove the carbohydrate from the column prior to applying another sample. Non-specifically bound material may become more difficult to remove if it is allowed to remain on the column for extended periods. Prior to storage, the lectin gel should be washed with 10ml of 1.4M NaCl prepared in distilled water. Re-equilibriate the column with the recommended buffer prior to storage.

DO NOT store the column in the high salt solution.

Storage

Microbial growth should be inhibited by the addition of 0.05% sodium azide (1mg/1ml). The buffers provided in the kit already contain 0.05% sodium azide as a preservative. 0.05% sodium azide should be added if another buffer is used for storage. Each lectin column must be stored with a small amount of solution to prevent drying of the matrix. The column must be capped at both ends to prevent desiccation during storage.

Special Warnings and Precautions

Refer to the enclosed MSDS for information. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

Calcium is REQUIRED for binding. 0.5mM Calcium is the maximum concentration in Buffer that will not form a white precipitate.

References

1. Eylar, E. H., et al. (1969) Immun. Lett. **22** : 13-16.
2. Iglesias, J. L., et al. (1982) Eur. J. Biochem. **123** : 247-252.
3. Debray, H., et al. (1986) Carbohydr. Res. **151** : 359-370.
4. Teneberg, S., et al. (1994) J. Biol. Chem. **269** : 8554-8563.
5. Lis, H., et al. (1985) Phytochemistry **24** : 2803.
6. Crowley, J. F. and Goldstein, I. J. (1981) FEBS Lett. **130** : 149-152.
7. Lis. H. and Sharon, N. (1987) Meth. Enzynol. **138** : 544.
8. Webb, C. W., et al. (1985) in "Cell Membranes and Cancer".
9. Galeotti, T., et al. Eds. Page 13. Elsevier, Amsterdam.
10. Baldus, S.E., et al. (1996) Int. J. Oncol. **9** : 43-48.
11. Harris, D.T., et al. (1987) J. Leukocyte Biol. **42** : 163-170.

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Reorder Information

The immobilized lectins may be purchased separately. The elution carbohydrates may be purchased as a set or individually. See catalog table, below.

Reorder Information

The immobilized lectins may be purchased separately. The elution carbohydrates may be purchased as a set containing all five carbohydrates.

Description	Cat. No.	Package Size
ECA Gel	A-5901-2	2ml
Carbohydrate Inhibition Kit	CIK-001	Set of 5 carbohydrates 0.1M α-Lactose 0.1M Melibiose 0.1M D-Mannose 0.05M N-Acetyl-D-glucosamine 0.05M α–L-Fucose

Additional Chromatography Products

In addition to more than 80 immobilized lectins, EY Laboratories, Inc. also manufactures a large selection of carbohydrate gels for lectin purification, antibody gels for purification of primary antibodies, and a number of different protein/glycoprotein gels. For further information, please contact customer service at EY Laboratories, Inc.

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MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006
Revision 4

PRODUCT IDENTIFICATION

Name: Proteins, carbohydrates, and biotin immobilized on a support matrix of acrylamide or polygalactose.

Catalog Number (s): ABP-01, A-1102 to A-9000, MB-1104 to MB-9000, PB-1104 to PB-9000, PG-001 to PG-7011, PB-01 to PB-05, CG-001 to CG-092, AG-001 to AG-032, A-1001 to A-1004, CG-094 to CG-096.

Synonyms: Protein A, Avidin (egg white), D-Biotin, Lectins, Secondary Antibodies, Carbohydrates, Thyroglobulin, Fetuin, Hemoglobin, α-Lactalbumin, Porcine Stomach Mucin, Ovalbumin, Bovine Submaxillary Mucin, Transferrin, Myoglobin, Strept. Avidin, and 2-Iminobiotin immobilized on a polygalactose matrix or an acrylic (or polyacrylaminde) matrix.

EMERGENCY INFORMATION

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

HAZARDOUS COMPONENTS

Specific protein(s) as listed on the vial label. The matrix itself is not known to be hazardous. The proteins are covalently attached to the beaded matrix and therefore present a hazard primarily through ingestion or injection. The biological activities of these chemicals will vary. It is possible that the immobilized material may leach off the beaded matrix during use. Care should be used when handling any of these reagents. All of these solutions contain at least 0.1%, but not greater than 1%, 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: No effects of overexposure have been documented. The individual proteins and other ligands may cause allergic reactions in sensitive individuals. This is a problem primarily with material that leaches from the column through use. Local irritation is likely if eye contact occurs.

ROUTES OF EXPOSURE: Ingestion or injection of the beaded material are the primary routes of exposure. Contact with the eyes may also present a hazard.

PHYSICAL CHARACTERISTICS

APPEARANCE: Solution containing a maximum of 50% (v/v) of beaded matrix in buffer.
SOLUBILITY: Not applicable.

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: All solutions contain less than 1% sodium azide (w/v) 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, 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 but it is recommended to handle these reagents in a fume hood when possible.

EYE PROTECTION: Required. Goggles or safety glasses with a side shield are Recommended.

RESPIRATORY PROTECTION: Not required unless the formation of aerosols is likely. 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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