Lectins Application and Resource Guide

Unlock deeper biological insights with glycobiology

vector LABORATORIES

Available in Canada from...

MJS BioLynx INC.

1-888-593-5969 • www.biolynx.ca • tech@chromspec.com

Bound to discover more

From tumor progression to viral capture to drug discovery, scientists are exploring the role of glycan modifications in normal and disease states. Lectins are the key to help you profile, characterize, and capture complex glycans in biological systems or leverage functional assays to ask questions that were previously beyond reach. Founded on a growing portfolio of purified lectins and lectin conjugates, Vector Laboratories supports the ability to profile, characterize, and capture complex glycans in biological systems.

Table of Contents

Lectins Introduction, Overview and History

Lectins—History

Lectins, named from the Latin legere (to choose), are now understood to be a broad class of glycan binding proteins that have their origin as plant proteins known to agglutinate red blood cells. Typically, lectins are named for the organism from which they are purified, with many retaining and interchangeably using the 'agglutinin' moniker and acronym. In general, a lectin is called 'Genus species lectin/agglutinin' e.g., *Erythrina cristagalli* lectin/*Erythrina cristagalli* agglutinin (ECL/ ECA) or *Sambucus nigra* agglutinin (SNA). See also the Table of Lectin Properties beginning on page 30 for lectin names and acronyms.

These hemagglutinins, or phytoagglutinins due to their plant derivation, were first described in 1888 by Peter Stillmark (Ref. 52). He isolated a potent toxin and hemagglutinin from castor bean seeds (*Ricinus communis* agglutinin, RCA). RCA was also used by Paul Ehrlich to make significant early immunological discoveries. By injecting mice with repeated small doses of RCA, he showed specific inactivation of the RCA toxin and that resistance can be conferred to the offspring of a treated mother.

These experiments were early foundations for antibody response specificity, immunological memory, and mother-child immunity transfer. James B. Sumner, an expert in protein isolation, isolated a lectin from jack beans, which he named Concanavalin A (Con A) in 1919. He was the first to crystalize an enzyme in 1926, an effort for which he was awarded a Nobel prize in 1946.

Years after his isolation of Con A, he discovered that it was possible to agglutinate erythrocytes in a process inhibited by sucrose, prompting the hypothesis that this lectin was interacting with the cell surface carbohydrates.

Another important step for lectins in immunology was in 1960 when Peter C. Nowell showed that phytohemagglutinin (PHA) was capable of inducing mitosis in lymphocytes. This discovery made the expansion and culture of lymphocytes *in vitro* possible for the first time. A finding in 1963 by Joseph C. Aub showed that wheat germ agglutinin (WGA) preferentially agglutinated malignant cancer cells, providing early evidence of altered glycosylation present in cancer (Ref. 59).

By introducing the concept of affinity chromatography for lectins by purifying Con A over dextran in 1967, Goldstein and Agrawal are credited with expanding the availability of purified lectins.

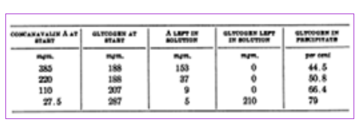
Until 1974, lectins had only been shown to be present in plants, invertebrates, and lower vertebrates. During 1974, Ashwell and Morell demonstrated the presence of the first mammalian lectin, the Ashwell- Morell Receptor (AMR), which is present in the liver and influences the half-life of glycoproteins and cells in circulation (Ref. 60). The following year, Vivian Teichberg isolated the first β-galactose lectin from the electric eel, and discovered the galectin family of proteins. These highly immunomodulatory molecules are still actively investigated today (Ref. 57).

Lectins Timeline

1888
Peter Hermann Stillmark
Describes lectins.

1890s
Paul Ehrlich shows immue neutralization of toxic lectins.

1919
Nobel laureate, James B Sumner, isolates Con A from jack bean.



CONCANAVALIN A AT START	GLYCOGEN AT START	A LEFT IN SOLUTION	GLYCOGEN LEFT IN SOLUTION	GLYCOGEN IN PRECIPITATE
mgm.	mgm.	mgm.	mgm.	per cent
385	188	153	0	44.5
220	188	37	0	50.8
110	207	9	0	66.4
27.5	287	5	210	79

1936
Sumner and Howell show:
- Con A agglutinates RBCs,
- Con A has sugar specificity

and hypothesize that it is due to carbohydrate binding.

1960
Peter C. Nowell shows PHA is a mitogen, allowing for lymphocyte expansion.

1963-65
Joseph C. Aub finds WGA preferentially agglutinates malignant cells. Uncovers, early evidence of altered glycosylation in cancer.

1967
Goldstein and his student (Agrawal) introduce affinity chromatography for lectins, increasing their purity, number, and availability.

The first evidence of the physiological role of plant lectins came from Irvin E Liener in 1976, when he reported that feeding beetles with a black bean lectin resulted in the death of beetle larvae. This insecticidal action of a lectin was found true for other lectins such as WGA, *Galanthus nivalis* lectin (GNL)", and Jacalin. In the words of Sharon and Lis ". . .lectins have come a long way since their first detection in plants as hemagglutinins to their present status as ubiquitous recognition molecules with myriad exciting functions and applications" (Ref. 52).

Lectins—applications

Lectins from plants and fungi are a defensive mechanism of these species to keep out invading proteins, cells, and organisms. These proteins have evolved to preferentially recognize carbohydrate structures, including those found on mammalian cells and tissues. Once purified, the specificity of each lectin can be harnessed as a tool utilized to affect and probe the complex glycans of biological systems.

Myriad of applications

Certain lectins have been suggested to be effective as promising agents for control of insect pests. Their incorporation into crops may decrease the amount of chemical agents needed for agriculture (Ref. 62). Others have been shown to have antifungal properties (Ref. 63).

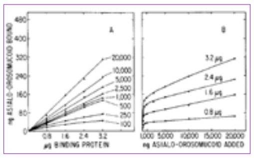
Altered glycosylation is a property of cancer cells, and many plant lectins have shown promising anticancer effects on cancer cells *in vitro* (Ref. 64).

Plant lectins have also been shown to have antiviral properties, suppressing the growth and preventing attachment of virions of coronavirus and HIV (Ref. 65).

The binding of lectins to HIV and their inhibitory effects have been increasingly explored, with many reports showing that lectin-gp120 glycan interactions resulted in inhibition of viral fusion (reviewed by Lam and Ng, Ref. 64). Lectins derived from bananas (BanLec) have also been demonstrated as potent inhibitors of HIV replication (Ref. 66). Another reported use of a plant lectin utilizes GNL to purify custom glycopeptides that mimic gp120 in order to immunize and elicit HIV neutralizing antibodies (Ref. 10).

In this guide, we review examples of techniques that utilize lectins in immunohistochemistry (IHC), immunofluorescence (IF), flow cytometry and cell sorting (FACS), affinity chromatography, ELISA, western blotting, surface plasmon resonance (SPR), cell proliferation, neural tracing, and *in vivo* perfusion. These are but a sample of the possible ways in which lectins are utilized in research applications; as protein-based tools to probe for glycans, while other applications merely await innovation, and development from imaginative investigators.

The Essentials of Glycobiology (Ref. 67), which includes a chapter on "Glycan-Recognizing Probes as Tools", is an excellent source of general information on glycoscience. We also recommend a review on lectin applications from Dan et al., (Ref. 68).



1974
Ashwell and Morell isolate the first mammalian lectin (now called the Ashwell-Morell Receptor — AMR).

1975
Vivian Teichberg isolates the first of the galectin family of proteins.

1976
Vector Laboratories, Inc. is founded on a growing portfolio of purified lectins and lectin conjugates which helped to pioneer lectin his to chemistry.

1976
Irvin E. Liener describes the role of plant lectins as protection from insect seed predators.

2004
"...lectins have come a long way since their first detection in plants as hemagglutinins to their present statusas ubiquitous recognition molecules with myriad exciting functions andapplications."
— Nathan Sharon and Halina Lis

Timeline References

Sumner and Howell (Ref. 58);
Aub (Ref. 59); Morell (Ref. 60);
Adapted from Sharon and Lis (Ref. 52).
Additional information: (Ref. 61).

Histology (IHC)

Histology—Immunohistochemistry

When seeking to observe the structures of cells and tissues, microscopy combined with a myriad of stains and dyes have been scientists' go-to techniques for hundreds of years. When enzyme-linked antibodies were introduced to this system, the location of specific proteins could be overlaid on top of the gross histology. Now, for those interested in carbohydrate structures, Vector Laboratories offers a library of lectins and lectin conjugates compatible with IHC. These lectins have enabled studies of influenza virus binding to human and animal tissues (Refs. 12 and 31), the use of lectins as cancer prognostics and diagnostics (Ref. 42), and as a marker for pathogenic infection (Refs. 26 and 43), as just a few examples.

Lectins are compatible with both Formalin-Fixed Paraffin-Embedded (FFPE) and frozen sections; however, there is a loss of some epitopes (mucins, glycolipids) during the FFPE preparation, thus the two systems may not yield equivalent results (Ref. 69).

Please see our Immunohistochemistry Guide for additional details.

Select Published Applications

- *Cancer diagnostic and/or prognostic (Ref. 42)*
- *Detection of pathogens (Ref. 43)*
- *Tissue epitope mapping for interaction studies (Ref. 31)*
- *Glycan alterations in pathological processes (Ref. 36)*

Procedural Overview

See Ref. 42 for a highly detailed Lectin IHC method

Prepare Slide for Staining

- Use the ImmEdge® Hydrophobic Barrier Pen to isolate sample and limit reagent usage.

Antigen Retrieval

- Variety of techniques using pH or enzymatic based retrieval solutions.

Block

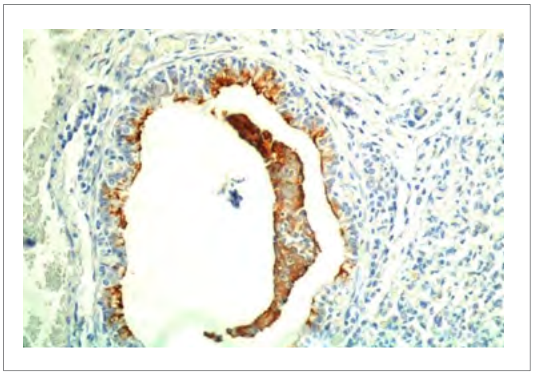
- Incubate slides for 30 minutes at room temperature in Carbo-Free™ Blocking (CFB) Solution.

Detection

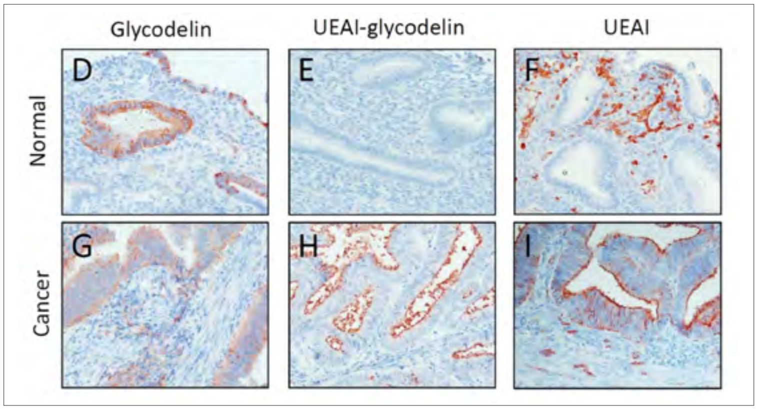
- Incubate slide with biotinylated lectin for 1 hour at room temperature, in CFB Solution. Suggested initial working range is 0.5 – 10 g/ml, user optimization required.
  » Alternatively, probe overnight at 4°C
- Wash slide with TBS with gentle agitation for 5 minutes—**perform three times (x3).**
- Apply streptavidin enzyme conjugate or VECTASTAIN® ABC enzyme reagent for 30 minutes at room temperature.
- Wash slide with TBS (5 minutes)—**perform three times (x3).**

Visualization

- Apply appropriate enzyme substrate following procedural guidelines for color development and target visualization.
- Coverslip specimen and view.



Lectin staining of bovine lung during bacterial infection. Biotinylated DBA (Fig. 2, Ref. 43).



In situ proximity ligation staining utilizing a lectin for detection of endometrial carcinoma. Biotinylated UEA I (Fig. 3, Ref. 98).

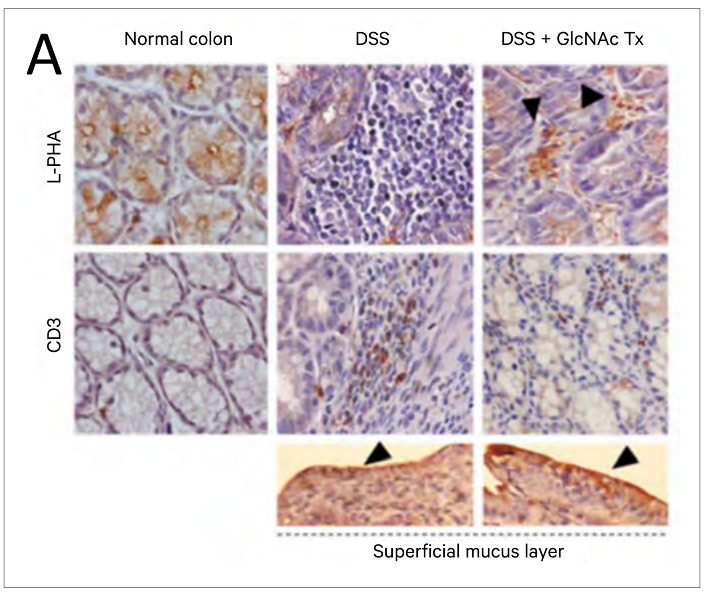
General Immunohistochemistry Workflow

Depending on the intended detection and visualization methodology, various reagent options are available throughout the workflow.

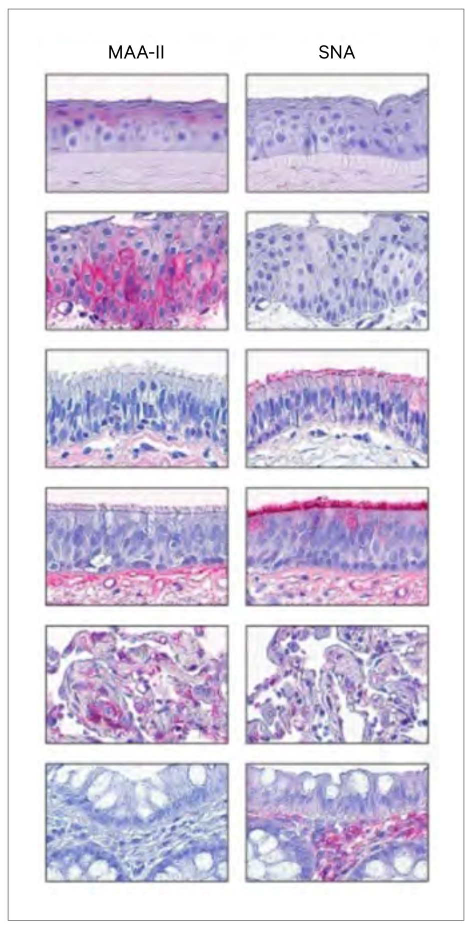
Tissue Preparation	Antigen Retrieval	Quench/ Block	Primary Antibody/ Lectins*	Secondary Antibody	Tertiary Reagent	Substrate/ Chromogen	Counterstain	Coverslip/ Mount	Visualize
• VECTABOND® Tissue Section Adhesive • ImmPrint® Histology Pen • ImmEdge® Hydrophobic Barrier Pen	• Antigen Unmasking Solutions, Citrate- or Tris-based	• BLOXALL® Endogenous HRP and AP Blocking Solution • Avidin/Biotin Blocking Kit • Streptavidin/Biotin Blocking Kit • Normal Sera • Animal-Free Blocker® and Diluent • BSA • Casein Solution • M.O.M.® (Mouse on Mouse) Blocking Reagent • Carbo-Free Blocking Solution	• Biotinylated Lectins • Unconjugated Lectins	• ImmPRESS® Polymer Reagents (HRP or AP) • ImmPRESS PLUS Polymer Reagents (HRP) • ImmPRESS Duet Polymer Reagents (HRP/AP) • M.O.M. (Mouse on Mouse) ImmPRESS Polymer Kit • M.O.M. (Mouse on Mouse) Basic Kit • Biotinylated Secondary Antibodies • Unconjugated Secondary Antibodies • Enzyme-Conjugated Secondary Antibodies (HRP or AP) • Biotinylated Anti-Lectins	• VECTASTAIN ABC-HRP Reagents • VECTASTAIN Elite® ABC-HRP Reagents • VECTASTAIN ABC-AP Reagents • VECTASTAIN Elite ABC-HRP PLUS Kit • ImmPRESS Excel HRP Amplified Staining Kits • M.O.M. (Mouse on Mouse) Elite Kit (HRP) • Enzyme-conjugated avidin/streptavidin (HRP or AP)	• HRP substrates • AP substrates/ Levamisole Solution	• Hematoxylin • Methyl Green • Nuclear Fast Red	• VectaMount® Express Mounting Medium • VectaMount Mounting Medium • VectaMount AQ Mounting Medium	

* For more information visit: vectorlabs.com/glycobiology

Note: HRP (Horseradish Peroxidase) and AP (Alkaline Phosphatase)



Monitoring tissue glycosylation state during an inflammatory model of colitis and treatment. (Fig. 5A, Ref. 99).



Lectin staining of human tissues for sialic acids. Biotinylated SNA; Biotinylated MAL II (Fig. 2, Ref. 31).

Histology (IF)

Histology—Immunofluorescence

Instead of using a chromogen to impart color to a cell or tissue section, immunofluorescence typically utilizes different fluorophores to visualize specific targets. By carefully selecting excitation and emission wavelengths for each reagent to prevent spectral overlap, it is possible to apply multiple probes to a tissue section. Vector Laboratories offers lectins conjugated to traditional and contemporary dyes for spectral color choices in single and multiplexed applications. When combined with antibody detection, such multiplexed microscopy allows visualization of overlapping elements such as a protein of interest and a specific glycan. An added feature of IF microscopy is the ability to evaluate relative amounts of fluorescence from each channel enabling comparison between samples with identical acquisition settings.

To further identify the different cell types that result from the differentiation of human embryonic stem cells, Dodla et al., employed a panel of eight lectins. By using IF, they were able to demonstrate that *Vicia villosa* lectin (VVL/VVA) could differentiate human neural progenitors from mesenchymal progenitors and embryonic stem cells—evidence that lectins can be tools for isolating of distinct cellular populations along differentiation pathways (Ref. 45).

Please see our Immunofluorescence Guide for additional details.

Select Published Applications

- *Visualize abnormal glycosylation compartmentalization in cancer (Ref. 11)*
- *Characterize cell subpopulations by unique lectin binding patterns (Ref. 45)*
- *Quantitate the change in glycan structure due to enzyme loss/mutation (Ref. 39)*
- *Differentiate intracellular parasites from host cells (Ref. 19)*

Procedural Overview

Prepare Slide for Staining

- Use the ImmEdge Hydrophobic Barrier Pen to isolate sample and limit reagent usage.

Antigen Retrieval

- Variety of techniques using pH or enzymatic based retrieval solutions.

Block

- Incubate slides for 30 minutes at room temperature in CFB solution.

Detection

- Incubate slide with fluorophore-conjugated lectin for 1 hour at room temperature, in CFB solution. Suggested initial working range is 0.5–10 g/ml, user optimization required. For additional amplification, a biotinylated lectin can be applied followed by a streptavidin fluorophore conjugate.
  » Alternatively, probe overnight at 4°C
- Wash slide with TBS with gentle agitation for 5 minutes—**perform three times (x3)**.

Quench Autofluorescence

- We recommend Vector® TrueVIEW® Autofluorescence Quenching Kits (SP-8400 or SP-8500).

Visualization

- If using a biotinylated lectin, the streptavidin fluorophore conjugate would be applied at this point.
- Wash slide with TBS with gentle agitation for 5 minutes—**perform three times (x3)**.
- Gently wipe slide dry.

Mount/Coverslip

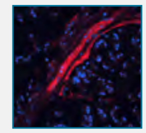
- Apply coverslip with VECTASHIELD® Antifade Mounting Media.

Signal Acquisition

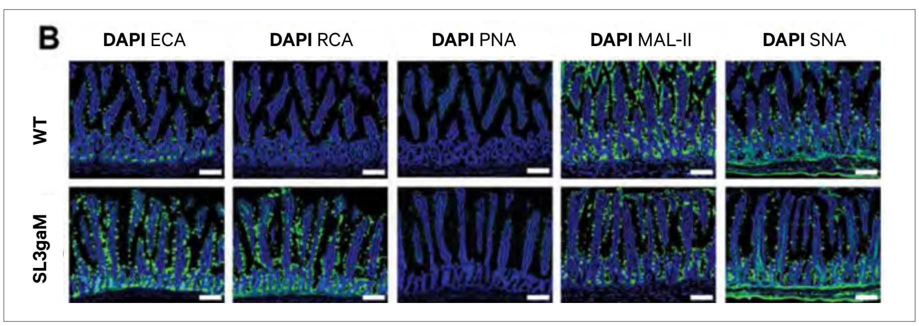
- Image capture via fluorescent microscopy.

General Immunofluorescence Workflow

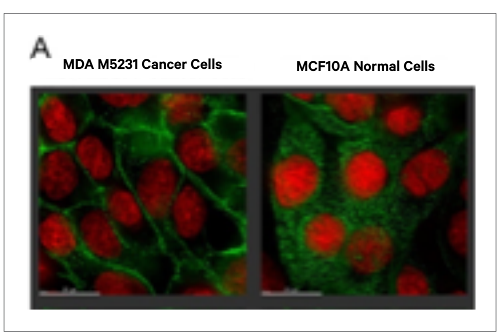
Depending on the intended detection and visualization methodology, various reagent options are available throughout the workflow.

Tissue Preparation	Antigen Retrieval	Quench/ Block	Primary Antibody/ Lectins*	Secondary Antibody	Tertiary Reagent	Counterstain/Mount	Visualize
• VECTABOND Tissue Section Adhesive • ImmPrint Histology Pen • ImmEdge Hydrophobic Barrier Pen	• Antigen Unmasking Solutions, Citrate- or Tris-based	• Vector TrueVIEW Autofluorescence Quenching Kits (with or without DAPI)** • Avidin/Biotin Blocking Kit • Streptavidin/Biotin Blocking Kit • Normal Sera • Animal-Free Blocker and Diluent • BSA • Casein Solution • M.O.M. (Mouse on Mouse) Blocking Reagent • Carbo-Free Blocking Solution • BLOXALL Endogenous HRP/AP Blocking Solution	• Biotinylated Lectins • Fluorochrome- Conjugated Lectins • Unconjugated Lectins	• VectaFluor™ DyLight™ R.T.U. Secondary Antibodies • VectaFluor Duet IF Double Labeling Kits • M.O.M. (Mouse on Mouse) Immunodetection Kits • Biotinylated Secondary Antibodies • Fluorochrome-Conjugated Secondary Antibodies • Unconjugated Secondary Antibodies	• VectaFluor Excel DyLight Amplified Fluorescent Staining Systems • Fluorochrome-Conjugated Avidin or Streptavidin • Biotinylated Anti-Avidin Amplifying Reagent • Biotinylated Anti-Streptavidin Amplifying Reagent	• VECTASHIELD Vibrance® Antifade Mounting Medium with or without DAPI counterstain (hard-setting) • VECTASHIELD PLUS Antifade Mounting Medium with or without DAPI counterstain (non-hardening) • VECTASHIELD Antifade Mounting Medium with or without DAPI counterstain (non-hardening) • VECTASHIELD Antifade Mounting Medium with Propidium Iodide (PI) (non-hardening) • VECTASHIELD HardSet Antifade Mounting Medium with or without DAPI counterstain (hard-setting) • VECTASHIELD HardSet Antifade Mounting Medium with TRITC—Phalloidin (hard-setting)	

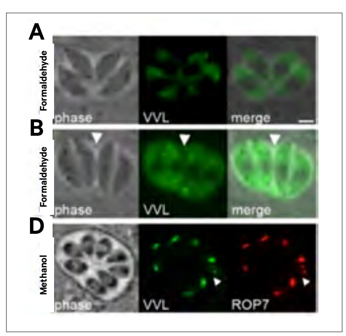
* For more information visit: vectorlabs.com/glycobiology
** TrueVIEW Autofluorescence Quenching is applied just prior to coverslipping



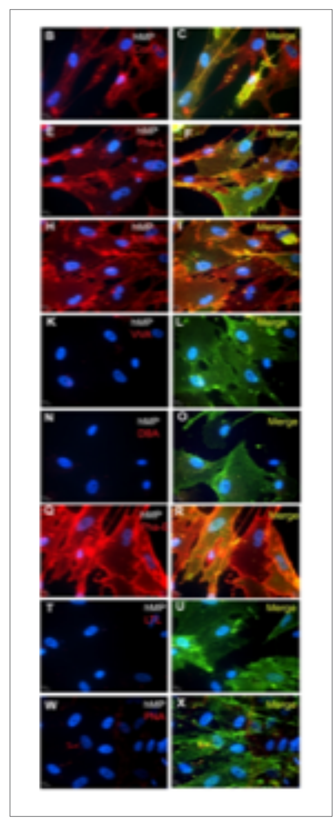
Glycosylation of murine small intestine. Biotinylated ECL/ECA; Biotinylated RCA; Biotinylated PNA; Biotinylated MAL II; Biotinylated SNA (Fig. S5, Ref. 39).



Compartmentalization of glycosylated structures. FITC MAL I (Fig. 1, Ref. 11).



Vicia Villosa Lectin staining in Toxoplasma. FITC VVL (Fig. 1, Ref. 19).



Lectin staining of human mesenchymal progenitor cells. Biotinylated Con A; Biotinylated PHA-L; Biotinylated MAL I; Biotinylated VVL; Biotinylated DBA; Biotinylated PHA-E; Biotinylated LTL; Biotinylated PNA (Fig. 6, Ref. 45)

Flow Cytometry (FC)

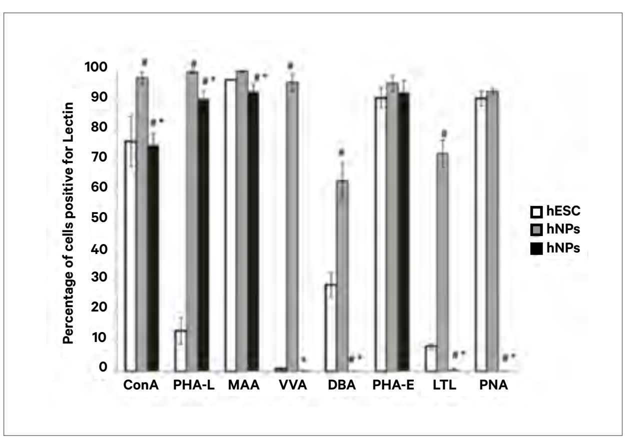
Flow Cytometry—Cell Analysis

When seeking to identify and compare cellular targets between cell populations, flow cytometry is a versatile platform capable of single to multiplex target analysis. In this technique, individual labeled cells are monitored for their fluorescent properties. When cells are incubated with antibodies and lectins conjugated to unique fluorophores, differentiation of cellular subtypes is made possible.

Lectin panels are an excellent way to monitor the status of cellular glycosylation, which is a prominent post-translational modification on cell surface proteins. Using lectins to probe for differential glycosylation states between cell populations is an effective strategy. It has been utilized to demonstrate that altered glycosylation is typical in cancer (Ref. 70) and may be useful in oncology diagnostics (Refs. 71 and 72). Cell surface glycosylation may also be indicative of cellular phenotype, such as platelet circulatory half-life (Ref. 73).

Select Published Applications for Cell Analysis

- *Differentiation of hematopoietic stem and progenitor cell stages (Ref. 50)*
- *Interrogation of cell surface receptor glycosylation (Ref. 17)*
- *Immune cell surface phenotyping (Ref. 37)*
- *Mutant cell line screening (Ref. 54)*



Characterization of lectin binding in human stem and progenitor cells. Biotinylated Con A; Biotinylated PHA-L; Biotinylated MAL I/MAA; Biotinylated VVL/VVA; Biotinylated DBA; Biotinylated PHA-E; Biotinylated LTL; Biotinylated PNA (Fig. 3, Ref. 45).

Procedural Overview

(Adapted from Ref. 4).

Sample Preparation

- Prepare single cell suspension, including any fixation and permeabilization steps, and red blood cell lysis.

Block

- CFB Solution can be used as a block and diluent if required.

Detection

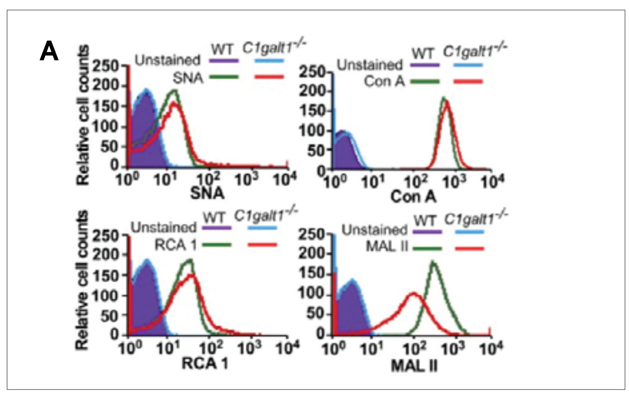
- Incubate cells with fluorophore-conjugated lectin for 1 hour at 4°C, in buffer. Suggested initial working range is 0.5–10 g/ml, user optimization required. Biotinylated lectins are also widely used for flow cytometry applications. See accompanying references and figure images.
- Wash cells with cold PBS—**perform two times (x2).**

Visualization

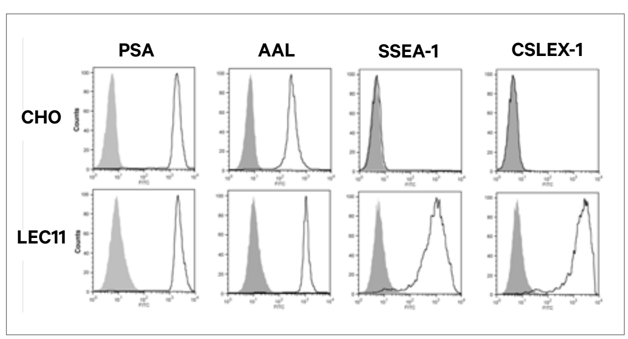
- If using a biotinylated lectin, incubate cells with streptavidin conjugated fluorophore at this step for 1 hour at 4°C in CFB Solution.
- Wash cells with cold PBS—**perform two times (x2).**
- Resuspend in PBS at desired concentration.

Signal Acquisition

- Acquire events from each sample.



Characterization of platelet cell surface glycosylation. Biotinylated SNA; FITC Con A; Biotinylated RCA; Biotinylated MAL II (Ref. 38).



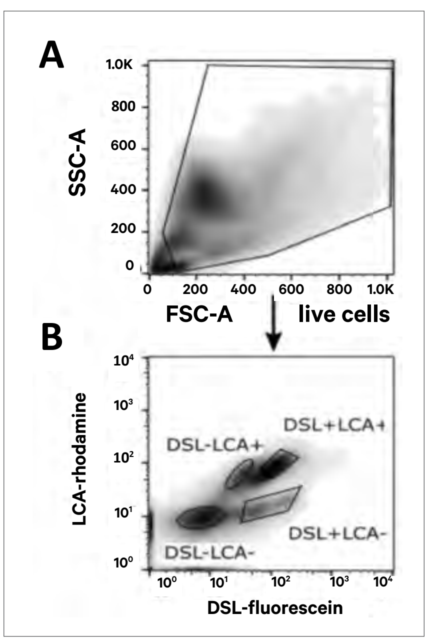
Characterization of CHO cell surface glycosylation. Biotinylated PSA; Biotinylated AAL (Ref. 54).

Flow Cytometry—Cell Sorting

Isolation of cell populations or even single cells as defined by cell surface markers is made possible by fluorescence-activated cell sorting. By staining cell populations with different fluorophores, unique cellular subpopulations can be identified and separated. This occurs when a desired cell is detected and subsequently diverted into its own container, either alone or with a similar population. For example, blood lymphocytes may be distinctly separated into FITC-CD4+ and PE-CD8+ populations. Inclusion of lectins in flow panels has led to further delineation of cell populations, resulting in new marker sets by which to identify human neural progenitor cells (Ref. 45). Use of lectins in a sorting strategy may also lead to greater enrichment of rare populations (Ref. 15). They may also be employed to differentiate subpopulations of cells, a useful strategy for clonal selection (Ref. 8).

Select Published Applications for Cell Sorting

- *Identification and characterization of novel cell types (Ref. 8)*
- *Enrichment of rare cell types (Ref. 15)*
- *Clonal selection of population (Ref. 49)*



Identification of novel cell populations based on lectin binding. Rhodamine LCA; FITC DSL (Fig. 6, Ref. 49).

Procedural Overview

(Adapted from Ref. 4).

Sample Preparation

- Prepare single cell suspension.

Block

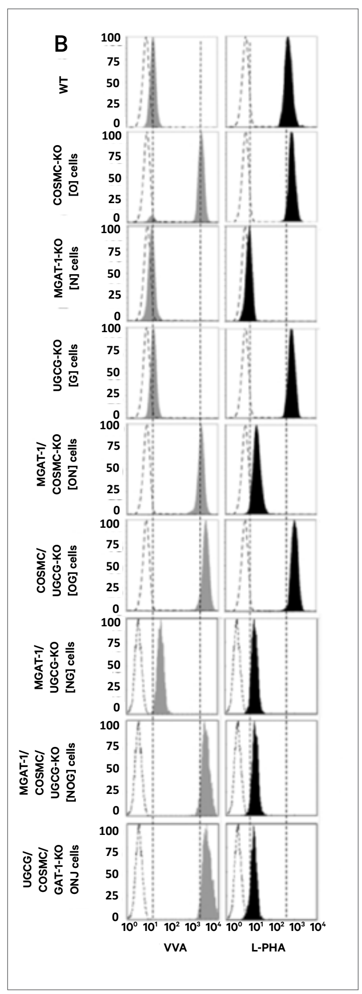
- CFB Solution can be used as a block and diluent if required.

Detection and Visualization

- Incubate cells with fluorophore-conjugated lectin for 30–60 minutes at 4°C, in CFB Solution. Suggested initial working range is 0.5–10 mg/ml. User optimization may be required.
- Wash cells with cold PBS—**perform two times (x2)**
- Incubate cells with different fluorophore-conjugated primary antibodies for additional sorting for 30–60 minutes at 4°C in CFB solution (if required).
- Wash cells with cold PBS—**perform two times (x2).**

Signal Acquisition

- Sort events from each sample.



Clonal sorting based on cell surface glycosylation. FITC-VVA; FITC-PHA-L (Fig. 2, Ref. 8).

Affinity Chromatography

Affinity Chromatography—Column or Batch

Affinity chromatography is a separation technique where molecules are selected through specific interactions with a solid phase substrate. For positive selection, molecules of interest bind to the column while all others are washed away. Bound molecules are then recovered after being eluted from the column. In negative selection, molecules of interest flow though the column, while others are retained. This can be used to enrich or purify specific glycoproteins, for example isolating a specific glycosylation variant of IgG, or separating glycosylated from non-glycosylated proteins in a sample.

Several lectins have known viral binding or antiviral properties; Pauthner et al., have capitalized on the HIV binding capacity of GNL to purify custom glycopeptides that mimic gp120 in order to immunize and elicit HIV neutralizing antibodies (Ref. 10). Other viral families are possible targets, as Con A, LCA, and PNA all have been shown to impact replication of the influenza virus (Ref. 74). For a comprehensive review of the antiviral properties of lectins, see Mitchell et al., (Ref. 75).

Select Published Applications

- *Purification of glycopeptides for vaccine development (Ref. 10)*
- *Enrichment for proteins with specific glycosylation patterns after media supplementation or knock-down treatment (Refs. 11 and 17)*
- *IgG enrichment for the protein fraction that contain sialic acid (Ref. 51)*
- *Identification of proteins that carry glycans of interest (Ref. 20)*

Procedural Overview

(Adapted from Ref. 4).

Sample Preparation

- Pipet agarose lectin slurry in to a column (i.e., inverted Pasteur pipette or commercial alternative) or dish format for the intended application.
- Prior to use, wash the agarose lectin resin thoroughly with buffer before use to remove preservatives and stabilizers.
- Wash buffers fortified with 0.1 mM Ca^{2+} and 0.01 mM Mn^{2+} can improve performance of some lectins.

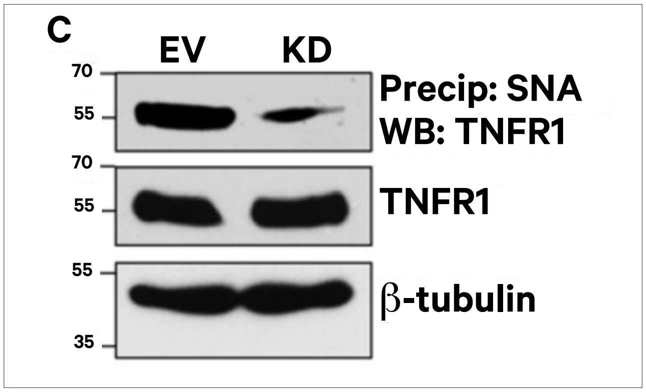
Incubate with agarose-bound lectin

For Batch

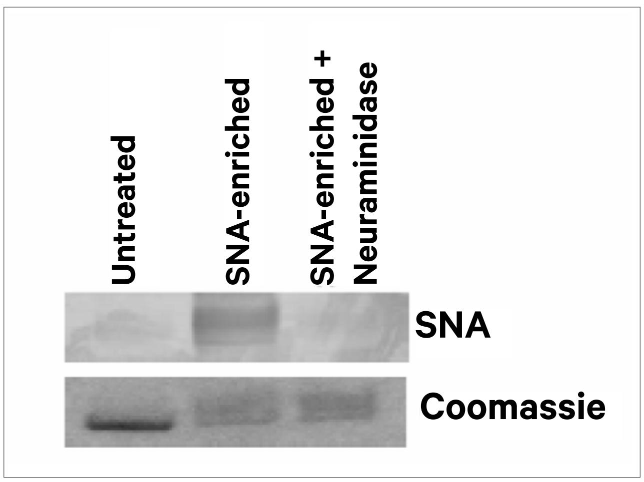
- Gently agitate sample with agarose lectin resin for 2–4 hours at room temperature.
  - » *Alternatively, incubate overnight at 4°C*
- Gently spin down beads (<1,000 g) and remove supernatant.
- Wash 3x with wash buffer.
- Incubate with 2x bead volume of appropriate eluting solution to your lectin column.
- Gently spin down beads (<1,000 g) and remove supernatant.

For Column

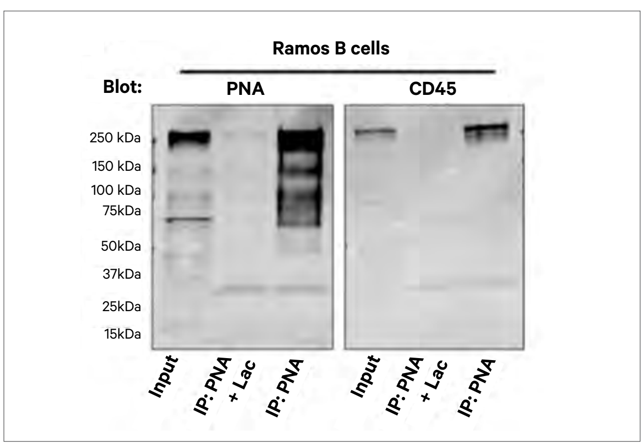
- Use gravity to slowly pull the glycoprotein sample through the column. Use of pressure will compress the beads and reduce binding. Depending on sample requirements, room temperature or cold room conditions can be applied.
- Wash resin with 2–3 column volumes of buffer to remove unbound material.
- Several column volumes of eluting solution may be required to achieve sufficient recovery.
- Wash and regenerate column.



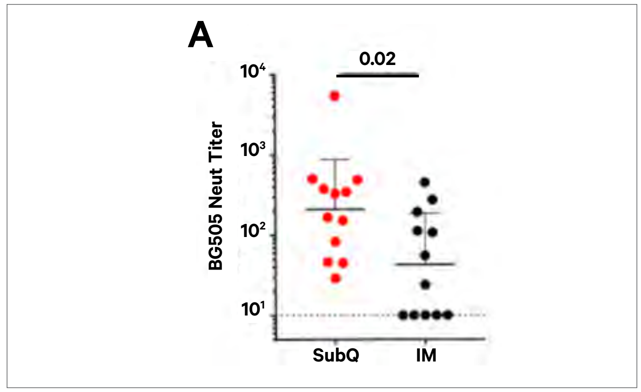
Determination of receptor glycosylation. Agarose SNA (Ref. 17).



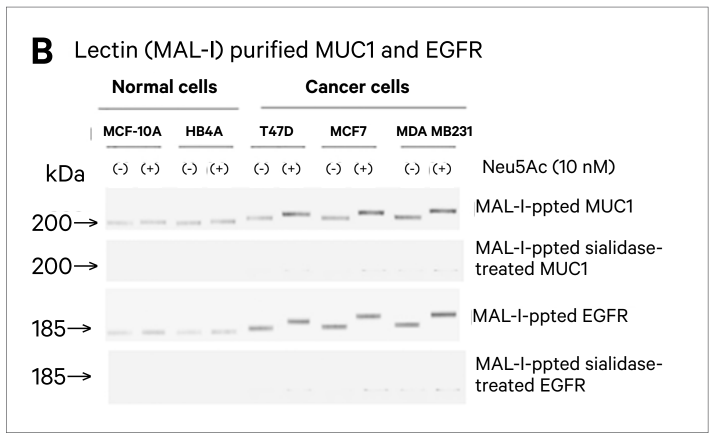
IgG enrichment for specific glycans via lectin chromatography. Agarose SNA (Sup. Fig 3, Ref. 51).



Glycan profiling of specific proteins by lectin affinity chromatography. Agarose PNA (Fig. 3E, Ref. 20).



Immune response to a HIV glycopeptide purified by lectin chromatography. Agarose GNL (Fig. 2, Ref. 10).



Examination of sialylation of MUC1 and EGFR on normal and malignant cells after sialic acid treatment under nutrient deprivation. FITC MAL I (Ref. 11).

ELISA

ELISA (Enzyme Linked Immunosorbent Assay)

ELISA is a robust technique that is typically performed in a plastic multiwell plate, and can be used to quantitatively detect target molecules. The technique is amenable for high-throughput systems utilizing 384-well plates and liquid handling devices. The specificity of a direct ELISA can be further enhanced by utilizing a sandwich ELISA technique, where a capture molecule is adsorbed to the plate instead of a sample. By properly designing both capture and detection agents so they do not bind the same region of the target, sensitivity and signal-to-noise ratios may be improved over standard ELISA techniques at the cost of additional reagents and steps. By changing capture or detection agents, targets and readouts can be quickly shifted in this adaptable system.

Dusowa et al., utilized a direct ELISA approach to characterize the glycan profile of extracellular vesicles derived from glioblastoma cell lines. They demonstrated that modulating the extracellular vesicle surface glycans resulted in increased uptake by dendritic cells, an important first step in presenting cancer antigens to the immune system (Ref. 18).

For glycan analysis of samples, we recommend starting with a panel of lectins as detection agents.

Select Published Applications

- *Screening protein glycosylation state (e.g., IVIG) (Ref. 3)*
- *Determining the glycans present on extracellular vesicles (Ref. 18)*
- *Utilized as a disease diagnostic or prognostic (Ref. 32)*

Procedural Overview

(Direct ELISA adapted from Refs. 3 and 34)

Sample Preparation

- Incubate sample in high-binding ELISA plates at 4°C overnight in recommended binding buffer.
- Wash plate with PBS plus Tween® 20 (PBST)—**perform four times (x4).**

Block

- Incubate plate for 60 minutes at room temperature in CFB Solution.
- Wash plate with PBST—**perform two times (x2).**

Detection

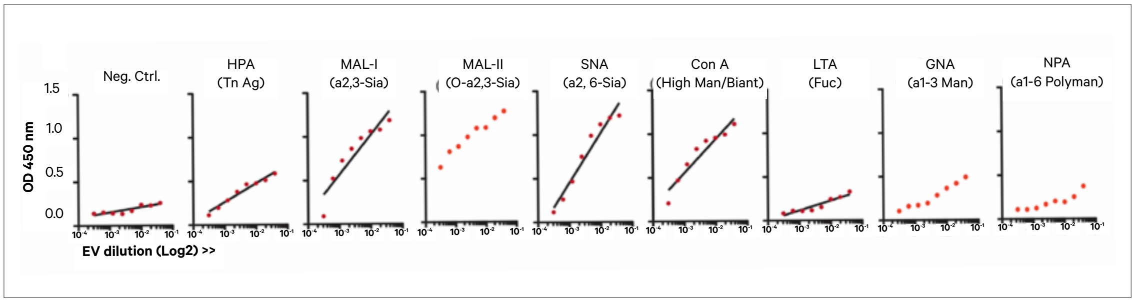
- Incubate plate with biotinylated lectin for 60 minutes at 4°C to room temperature (user optimized), in CFB Solution. Suggested initial working range is 0.5–10 mg/ml, user optimization required.
- Wash plate with PBST—**perform four times (x4).**

Visualization

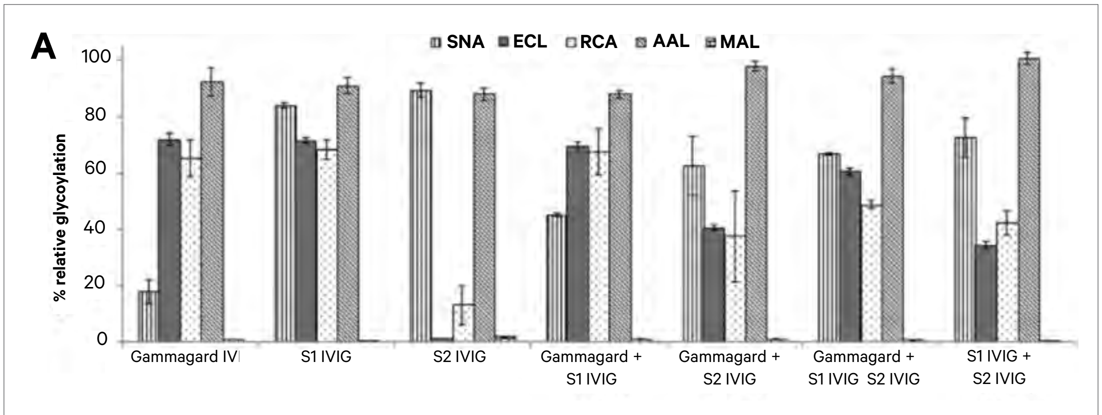
- Incubate plate with enzyme-linked streptavidin or avidin as per manufacturer's instructions.
- Wash cells with PBST—**perform four times (x4).**
- Apply appropriate enzyme substrate solution.

Signal Acquisition

- Read plate at the appropriate wavelength for the chosen detection system.



Surface glycosylation of glioblastoma extracellular vesicles by lectin ELISA. Biotinylated Con A; Biotinylated MAL I; Biotinylated MAL II; Biotinylated SNA; Biotinylated LTL; Biotinylated NPA (Fig 2, Ref 18).



Screening IVIG Fc fragment glycosylation via lectin ELISA. Biotinylated SNA; Biotinylated ECL; Biotinylated RCA; Biotinylated AAL; Biotinylated Con A; Biotinylated MAL II (Fig. 4, Ref. 3).

Abbreviation	Reaction with[b]	
	GdA	HEC-1B Gd
SNA	+++	–
ECL	–	+++
UEAI	–	+++
Con-A	+	+++
PSA	+	+
LCA	+	+++
RCA I	+	+
GSL II	–	–
GSL I	–	–
DBA	–	–
SBA	+	+++
SJA	–	–
VVA	–	–
WFA	+	++
WGA	+	++
WGA_{succ}	+	+++
DSL	–	–
LEL	–	–
STL	+	+
PNA	–	–
Jacalin	–	–
PHA-E	–	–
PHA-L	+	+

Characterization of recombinant glycodelin glycosylation by modified sandwich lectin ELISA immunoassay. Biotinylated Lectin Kit I, Biotinylated Lectin Kit II, Biotinylated Lectin Kit III (Table 1, Ref. 98).

Western Blotting

Western Blotting—Chemiluminescence

When isolating, identifying, or searching for glycoproteins, western blotting is a choice analytical technique. Proteins are separated by size via electrophoresis and transferred to a membrane for analysis. This procedure retains protein glycosylation, which can be detected by lectins. Detection methodologies vary for blotting applications. Here we present detection and visualization using a chemiluminescence approach with enzyme-based reagents.

While electrophoretic shift assays are commonly the first indication of a glycosylated target, probing with lectins can generate a more precise glycan profile. A variety of lectin screening kits are available for glycoprotein research (see page 38).

Select Published Applications

- *Whole sample protein glycosylation analysis (Ref. 47)*
- *Determine glycosylation of specific protein(s) (Ref. 30)*
- *Track glycosylation changes across physiological events (Ref. 28)*

Procedural Overview

Sample Preparation

- Prepare samples as per your own procedure.
- Perform gel electrophoresis and membrane transfer.

Block

- Incubate membrane for 30–60 minutes at room temperature in CFB Solution.

Detection

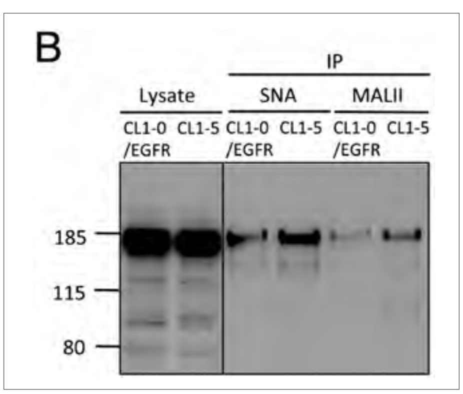
- Probe membrane with biotinylated lectin for 1 hour at room temperature, in CFB Solution. Suggested initial working range is 0.5–10 mg/ml, user optimization required.
- Wash membrane with TBST 5–10 minutes at room temperature—**perform four times (x4)**.

Visualization

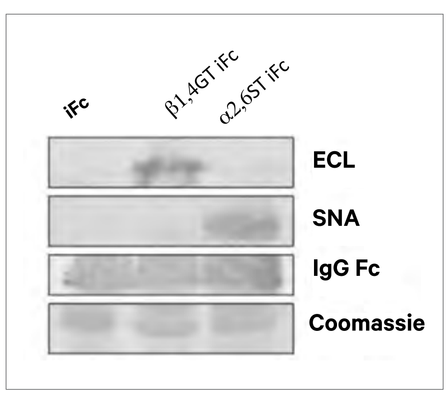
- Incubate membrane with streptavidin conjugated enzyme or VECTASTAIN ABC reagent for 1 hour at room temperature in CFB Solution. Vector Laboratories offers a wide variety of HRP and AP reagents to support your needs.
- Wash membrane with TBST 5–10 minutes at room temperature—**perform four times (x4)**.

Signal Acquisition

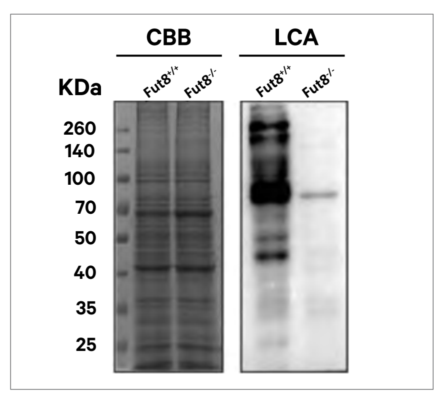
- Apply chemiluminescent or colorimetric enzyme substrate following procedural time lines.
- Use imager or film to capture image of membrane.



Detection of the sialylation state of EGFR. Biotinylated SNA; Biotinylated MAL II (Fig. S2, Ref. 100).



Lectin Blots of modified IVIG Fc fragments. Biotinylated SNA; Biotinylated ECL (Fig. 4, Ref. 30).



Loss of glycosylation leads to loss of T cell function (mouse splenic lysate). Biotinylated LCA (Fig. 2, Ref. 28).

Western Blotting

Western Blotting—Fluorescent Multiplexing

When isolating, identifying, or searching for glycoproteins, western blotting is a choice analytical technique. Proteins are separated by size via electrophoresis and transferred to a membrane for analysis. This procedure retains protein glycosylation, which can be detected by lectins.

By using fluorescent detection methods, multiple probes (carefully selected) can be used simultaneously on the same membrane, which provides the opportunity to accurately quantitate and compare all signals from each band of interest. Glycosylation status of a protein can be determined if a certain lectin binds within the protein band (co-localizes). A good example of this technique using fluorescence is provided in the figure from reference 14 that discerns IgG Fc fragment glycosylation from the Fab fragment. The adjacent procedure provides a brief overview of this methodology.

Select Published Applications

- *Overall glycan analysis of tissue or cell lysates (Ref. 4)*
- *Determination of IgG glycosylation (Ref. 14)*
- *Monitor specific protein glycosylation across cell processes (Ref. 20)*

Procedural Overview

Sample Preparation

- Prepare samples as per your own procedure.
- Perform gel electrophoresis and membrane transfer (a PVDF membrane is recommended for less background).

Block

- Incubate membrane for 30–60 minutes at room temperature in CFB solution.
  - » *Alternatively, block overnight at 4°C.*

Detection

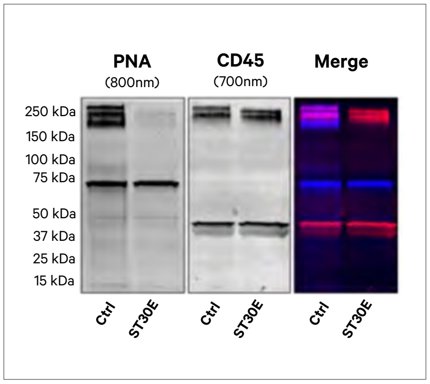
- Probe membrane with fluorophore-conjugated lectin for 1 hour at room temperature in CFB Solution. An alternative approach would be to use a biotinylated lectin. Suggested initial working range is 0.5–10 mg/ml, user optimization required.
  - » *Alternatively, probe overnight at 4°C.*
- Wash membrane with TBST 5–10 minutes at room temperature—**perform four times (x4)**.
- Probe membrane with fluorophore-conjugated primary antibody against protein of interest for 1 hour at room temperature in CFB solution.
  - » *Alternatively, probe overnight at 4°C.*

Visualization

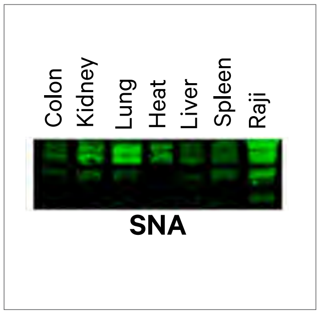
- If a biotinylated lectin was used incubate for 1 hour with fluorophore-conjugated avidin or streptavidin.
- Wash membrane with TBST 5–10 minutes, rocking, room temperature —**perform two times (x2)**.

Signal Acquisition

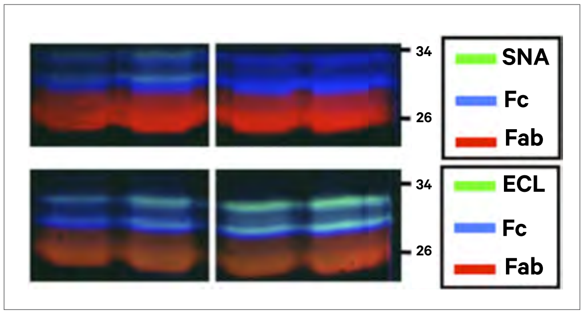
- Dry membrane.
- Image and analyze data.



Detection of differential protein glycosylation during cell maturation. Biotinylated PNA (Fig. 3E, Ref. 20).



Broad detection of tissue glycosylation from various wild-type mouse organs. FITC SNA (Ref. 4).



Detection of murine IgG Fc and Fab glycosylation during inflammation. Biotinylated SNA; Biotinylated ECL (Ref. 14).

Enzymatic Assays—Glycosyltransferases/Glycosidases

Enzymatic Assays—Glycosyltransferases/ Glycosidases

Glycosyltransferases and glycosidases often exist in low concentrations that are hard to directly detect. It is often more practical to determine the presence of a glycosyltranferase (an enzyme that adds a sugar) or glycosidase (an enzyme that removes a sugar) by measuring the change in either the enzyme's substrate or product. For glycosyltransferases, a decrease in the amount of acceptor and increase in the amount of enzymatic product is evidence of enzyme presence and activity. Conversely, loss of substrate implies the presence of a glycosidase. By employing lectins to track the relative amount of substrate and product, a variety of assays can be built to monitor enzymatic activity.

To elicit a site-specific anti-inflammatory response *in vivo*, Pagan et al., (Ref. 9) generated recombinant IgGs with an attached galactosyltransferase and sialyltransferase as a single macromolecule. They then tested whether the expressed protein retained enzymatic activity, by incubating it with donor nucleotide-sugars and a fetuin acceptor and monitored enzymatic product via lectin blotting.

Select Published Applications

- *Determine the activity of recombinantly expressed proteins (Ref. 9)*
- *Demonstrate the activity and specificity of bacterial enzymes (Ref. 24)*
- *Verify and validate genetic over- or underexpression systems (Ref. 20)*
- *Discerning enzymatic presence in novel systems (Ref. 44)*
- *Monitor glycosylation output after glycosyltransferase manipulation (Ref. 101)*

Procedural Overview

Sample Preparation

- Prepare the source of the enzyme in question.
- Prepare the substrate for the enzymatic reaction. This is commonly live or fixed cells, a glycoprotein in solution, a glycoprotein on a fixed surface, or purified complex carbohydrates in solution or fixed to a solid substrate.

Block

- To decrease non-specific interactions with your substrate, consider a blocking agent in your system. Incubate substrate for 30–60 minutes at room temperature in CFB Solution.
- Wash with two changes of a compatible wash buffer at room temperature—**perform two times (x2)**.

Reaction

- Add reaction buffer with enzyme source. Glycosytransferases will require a nucleotide-sugar donor, glycosidases will not. Both are highly sensitive to pH and cation concentration; optimize your reaction buffer accordingly.
- Incubate enzyme sample and substrate with gentle mixing. Time and temperature should be optimized by end user. We recommend 2–4 hours between room temperature and 37°C as a starting point.
- Separate reactant from source material if possible, by washing twice with a compatible wash buffer at room temperature.

Detection

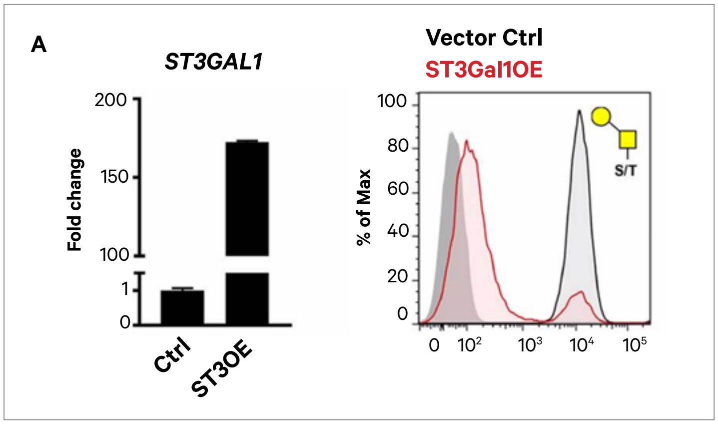
- Probe reactant with a fluorophore-labeled lectin for 1 hour at room temperature in CFB Solution. An alternative approach would be to use a biotinylated lectin.
  - » *Alternatively, probe overnight at 4°C.*
- Wash with a compatible wash buffer room temperature—**perform three times (x3)**.

Visualization

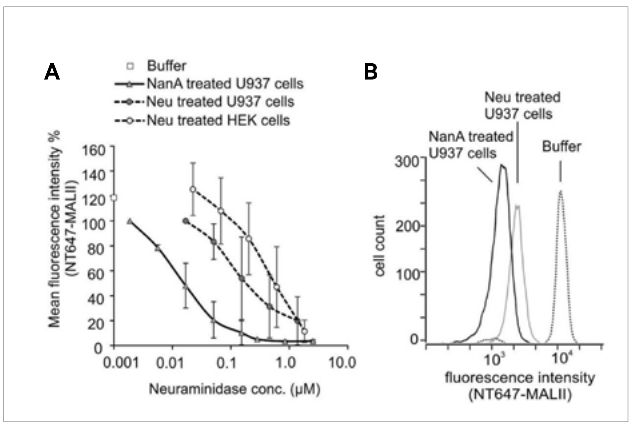
- If a biotinylated lectin was used, fluorophore-conjugated streptavidin would be applied at this point.
- Wash with a compatible wash buffer room temperature—**perform two times (x2)**.

Signal Acquisition

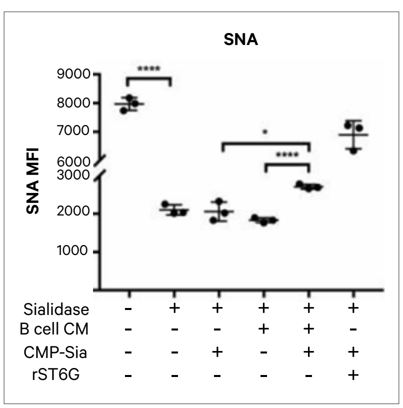
- Image and quantitate signal



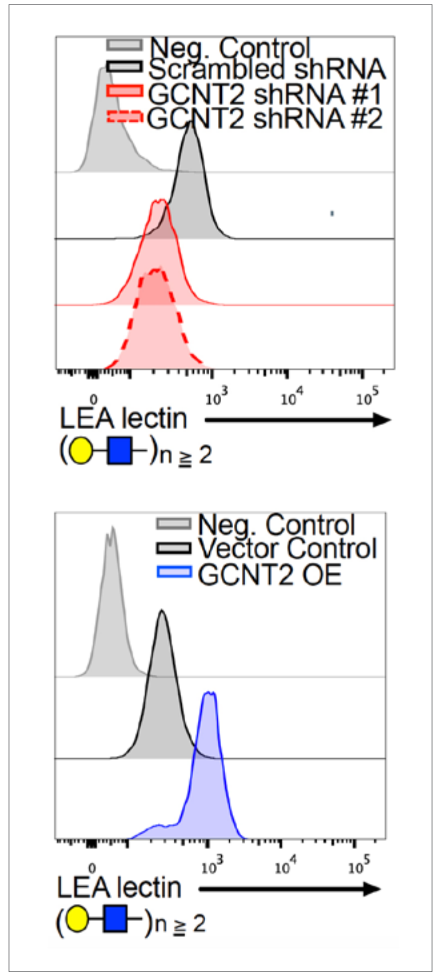
Loss of acceptor resulting from overespression of an active glycosyltransferase. Biotinylated PNA (Fig. 2, Ref. 20).



Loss of cell surface sialic acids from neuraminidase activity. Unconjugated MAL II (Fig. 1, Ref. 24)



Determination of enzyme activity by gain of cell surface glycosylation. FITC SNA (Fig. 2, Ref. 44).



Measurement of cell surface glycosylation resulting from glycosyltransferase knockdown and overexpression. (Ref. 101).

Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance (SPR)

When investigating molecular interactions, knowing the binding kinetics and the affinity constant for a given set of binding partners is of interest. When it is also important to perform such experiments with unlabeled components, e.g., if a fluorophore is too bulky to attach or is suspected to change binding kinetics, surface plasmon resonance may be the preferred technique. In short, a suspected ligand is affixed to a thin film of gold mounted to a slide which is backed by a prism. By directing polarized light at the prism and gold surface at a fixed angle, any molecular interaction with the ligand that increases the mass of the molecular complex will be detected by a change in the angle between the incident and reflected photons. This is typically carried out under flow conditions, thus enabling the real-time monitoring of the interaction between unlabeled pairs. For additional information on glycobiology and SPR, see the review by Duverger et al., (Ref. 76).

Select Published Applications

- *Discerning the glycans on a target molecule (such as antibodies) (Refs. 12 and 13)*
- *Probing the binding specificity or behavior of a lectin (Refs. 25 and 26)*
- *Disease specific biomarker and bioanalytic testing (Ref. 50)*

Procedural Overview

Sample Preparation

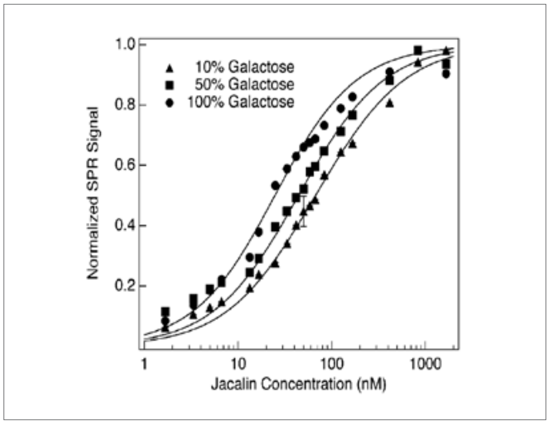
- Immobilize targets to chip with chemistry appropriate for your purpose.
- Wash the flow cell with running buffer e.g., PBS containing 0.005% polysorbate 20).

Block

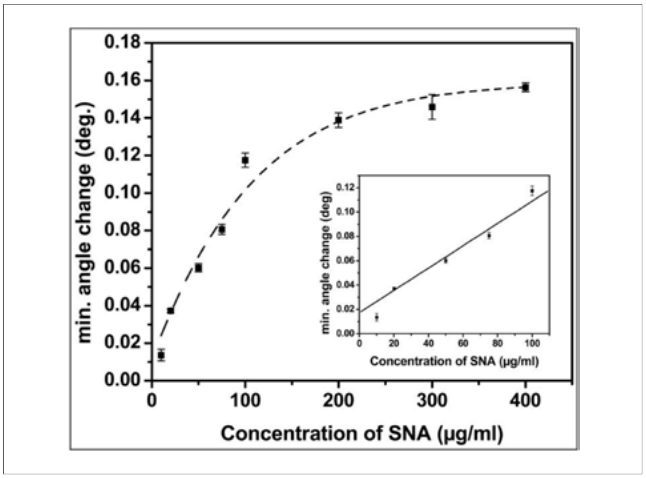
- Block the active flow chamber with 1.0 M ethanolamine/HCl, pH 8.5 for 10 minutes.
- Wash with running buffer.

Reaction and Detection

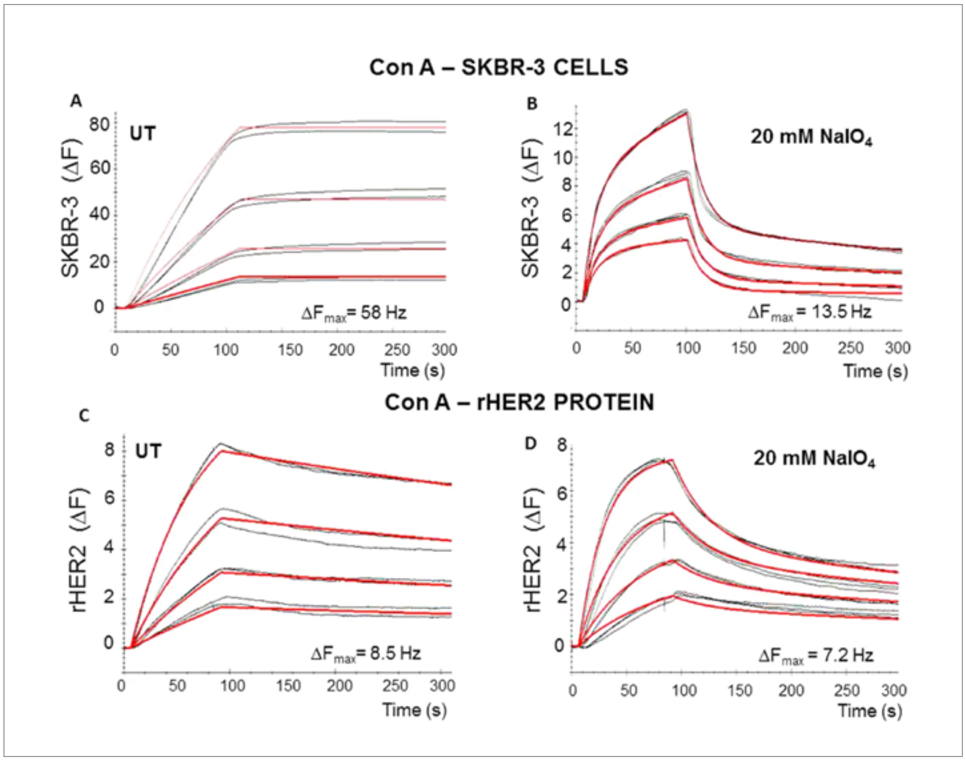
- Run unconjugated lectins across flow chamber at 10 L per minute for 3 minutes at room temperature (21–24°C). We suggest using CFB Solution as a sample buffer.
- Regenerate chip surface with 10 mM glycine pH 2.0 an 10 mM NaOH for 1 minute between each lectin concentration to remove any binding from the previous run.



Surface galactose composition characterization by Jacalin. Unconjugated Jacalin (Fig. 8, Ref. 26).



Characterization of SNA binding to a synthetic substrate. Unconjugated SNA (Fig. 4, Ref. 25).



Glycan characterization of recombinant antibodies. Unconjugated Con A (Ref. 102).

Cell Culture—Proliferation/Activation/Cytotoxicity

Cell Culture–Proliferation/Activation/Cytotoxicity

Cell surface proteins are commonly glycosylated, which facilitates molecular structure, solubility, and can modulate function. For proteins with intracellular signaling domains, direct engagement or clustering events can trigger a signaling cascade. These events can also be initiated by engagement of the molecule's carbohydrates, and exposure to plant and fungal lectins has been shown to have dramatic effects on cell behavior. The discovery, by Peter Nowell in 1960, that the lectin PHA could cause leukocytes to proliferate allowed for the first *in vitro* expansion of this cell type. Other lectins such as Con A can cause T cell activation and sensitivity to other growth signals (Refs. 77-79). In a study of T cell response to G-CSF, Nawa et al., used Con A stimulated CD3+ cells to measure proliferation and cytokine production (Ref. 6).

Lectins are not universally mitogenic; the same lectins that cause growth in leukocytes can cause cell death in other cells, such as CHO cells. This observation by Stanley et al., led them to develop additional mutant cell lines and propose a novel functional method of determining cell surface glycosylation via lectin cytotoxicity (Ref. 46). The aberrant cell surface glycosylation of cancer cells has led researchers to consider whether cytotoxic plant lectins could be used as antitumor molecules (Refs. 53 and 80). A brief review of the antitumor and mitogenic activity of mushroom derived lectins is provided by Hassan et al., (Ref. 81).

Select Published Applications

- *Lymphocyte proliferation and response to stimulation (Refs. 6, 7, and 109)*
- *Cell cytotoxicity (Ref. 46)*
- *Modulate cell growth and apoptosis in vivo with animal models (Ref. 48)*
- *Antitumor agent during in vitro and in vivo models (Ref. 53)*
- *Support growth of human pluripotent stem cells without differentiation (Ref. 56)*
- *Probing lymphocyte behavior from animal models (Ref. 103)*

Procedural Overview

T cell Proliferation

Assay

To a population of cultured human T cells, incubate with a range (e.g., 5–50 g/ml) of PHA-L or Con A in complete growth media to promote activities such as proliferation and activation. Optimal concentrations should be defined by the end user.

Note that Con A and PHA-L are selective mitogens and do not promote growth or proliferation. The assay overview provided here is an established model of mitogenic proliferation using these defined lectins. Using a known model system as a control provides guidelines when establishing insights into mitogenic activity for different lectins and compounds on mammalian cell types other than those mentioned above.

Assessment

Mitogenic stimulation can be assessed through several means, depending on the quantitative requirements of the study. Microscopic examination of phenotypic cell changes and colormetric assays assessing cellular enzyme activity have traditionally been used for semi-quantitative analysis. More quantitative means using flow cytometry, for example, are achieved through evaluation of protein and/or DNA synthesis and mitosis.

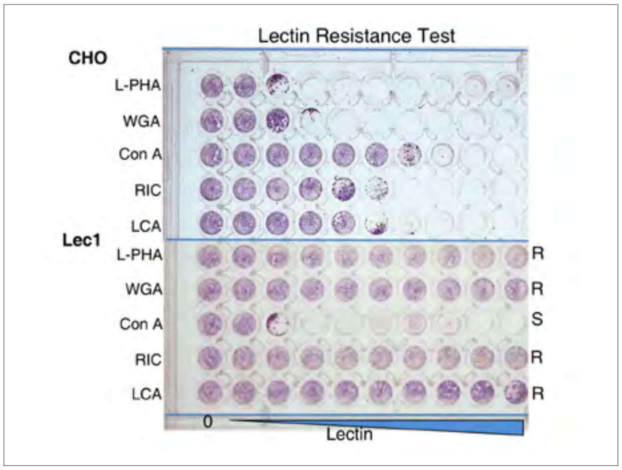
Cytotoxicity

Assay

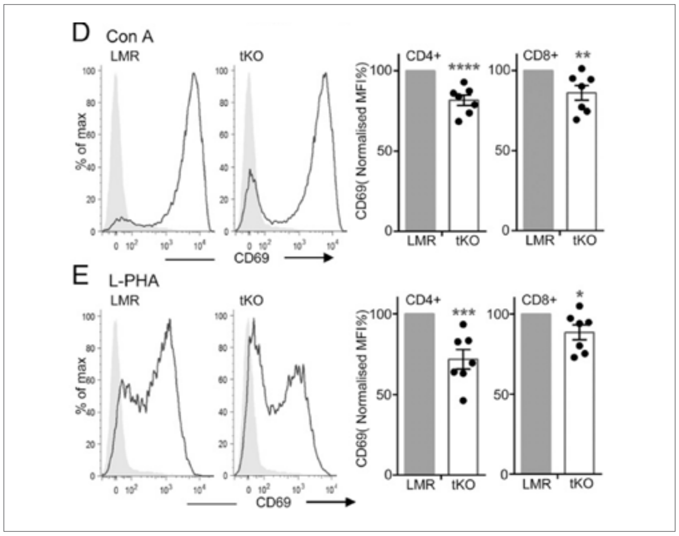
For a cell culture application, the methodology is fairly straightforward, whereby a specific lectin is added to the culture medium at varying concentrations and evaluated at multiple time points. Establishing a known model system of lectin toxicity against a given cell line will be helpful in streamlining workflow parameters for unknown lectin/cell interactions. The paper by Stanley (Ref. 46) provides a step-by-step protocol for setting up such an assay. The authors used Con A and PHA-L lectins in their study due to the known toxicity of these lectins toward CHO cells.

Assessment

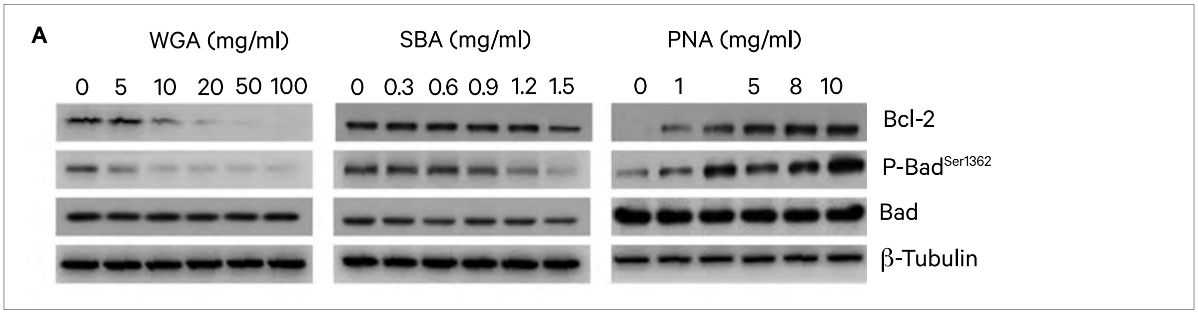
For lectin resistance type assays as outlined by Stanley (Ref. 46), toxicity was gauged by staining remaining live cell populations or by determining cellular enzyme activity. Depending on assay parameters, alternative procedures, such as the use of specific markers combined with platforms, could be used to detect cell death or apoptosis.



Lectin toxicity reveals altered cell surface glycosylation. Unconjugated PHA-L; Unconjugated WGA; Unconjugated Con A; Unconjugated RCA II; Unconjugated LCA (Fig. 2, Ref. 46, © 2014 John Wiley & Sons, Inc.)



Characterization of cell activation by stimulating splenocytes in Fng triple KO mice with lectins (D-E shown) and other agents (A-C not shown). Unconjugated PHA-L; Unconjugated Con A (Fig. 11D-E, Ref. 109). Originally published in The Journal of Immunology. Song, Y. et al., 2016. Lunatic, Manic and Radical Fringe Each Promote T and B Cell Development. 196:232-243. © 2015 The American Association of Immunologists, Inc.



Plant Lectin modulate markers of cell proliferation and apoptosis in zebrafish (Fig. 2, Ref. 48).

Neuronal Tracing

Neuronal Tracing

When exploring neuronal networking in the central nervous system, the use of lectins as neural tracers is a common practice. The directionality of axon transport can be harnessed by selecting the appropriate tracing molecule; from the soma to the synapse (anterograde) or from the synapse to soma (retrograde transport).

Retrograde tracing: The enzyme HRP was found to be an effective molecule as a retrograde tracer in 1971 (Ref. 82). It passes neuronal membranes non-selectively by passive endocytosis (Ref. 83). Conjugation of HRP was subsequently found to increase the rate of cellular uptake and transport (Ref. 84).

Anterograde + Retrograde tracing: When WGA binds to the glycans present on the neuronal plasma membrane; it is internalized by passive endocytosis and transported in both the anterograde and retrograde directions, providing extensive coverage of the neuron (Ref. 85). WGA can also be transported transsynaptically to other neurons in a network (Ref. 86).

Anterograde tracing: Among anterograde tracers, PHA-L is one of the earliest and most frequently used anterograde tracers (Ref. 84). Like WGA, PHA-L also binds to plasma membrane carbohydrates prior to cellular internalization.

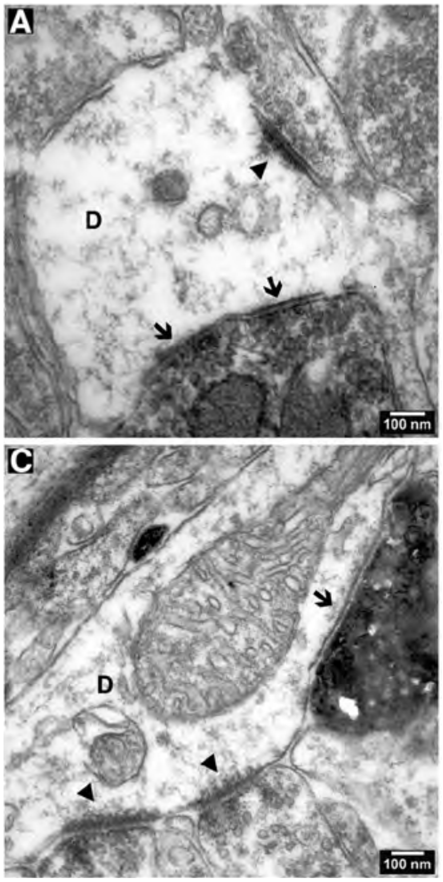
For a more comprehensive review of neural tracing techniques, see Vercelli et al., (Ref. 88).

Select Published Applications

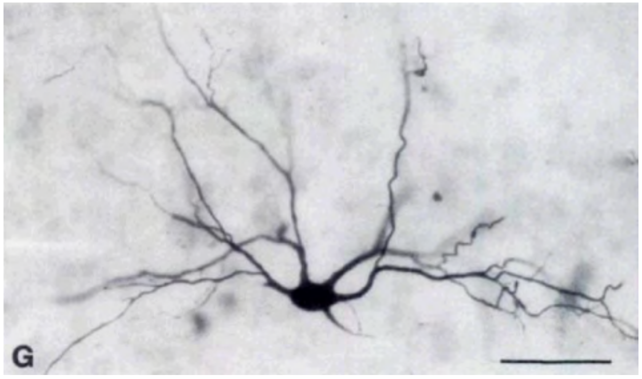
- *Visualization of anterograde neuronal transport (Refs. 1 and 2)*
- *Determination of neuron-cell interaction (Ref. 16)*
- *Tracking transneuronal transport of viral particles (Ref. 55)*
- *Observing neuron-neuron interactions (Refs. 34 and 111)*
- *Demonstrate specific protein expression of neurons (Ref. 35)*

Procedural Overview (Refer to Ref. 106)

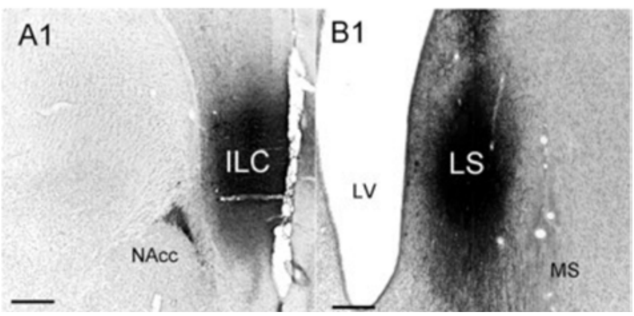
For PHA-L, we direct you to PHA-L Method for Tracing Efferent Neuronal Projections. Available on our website.



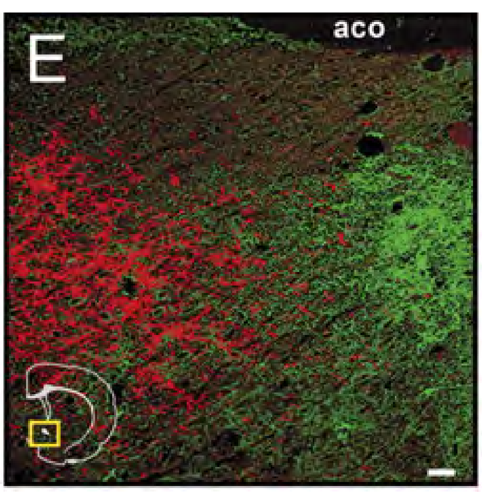
EM images of synapse formation in the CA1 region from PHA-L infused mice. Synaptic junctions indicated by arrows. Unconjugated PHA-L; Rabbit anti-PHA-(E+L); Goat anti-PHA-(E+L) (Fig. 7, Ref. 104)



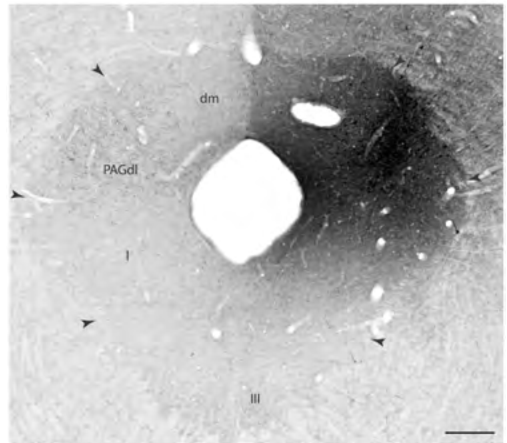
PHA-L labeling of ganglionic neuron of frog optic tectum. Unconjugated PHA-L (Fig 3G, Ref. 1).



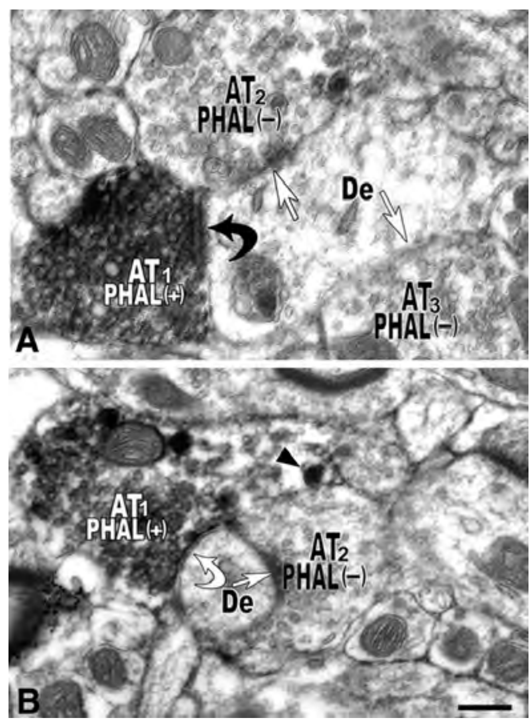
PHA-L labeling of the infralimbic vortex. Unconjugated PHA-L; anti-PHA-L (Fig. 6, Ref. 2).



PHA-L (red) and BDA (green) co-labeling of the substantia innominata. Unconjugated PHA-L; Goat anti-PHA-L, (Fig. 1, Ref. 105).



Neuronal labeling of an injection site at the dorsolateral column of the periaqueductal gray. Unconjugated PHA-L (Fig. 6, Ref. 106).



EM image of teh rat ventral tegmental ara (VTA) where a PHA-L labgeled axon terminal (AT1) forms asymmetric (A) or symmetric synapses (B) with a dendrite. Unconjugated PHA-L (Fig. 2, Ref. 111).

Vascular Perfusion

Vascular Perfusion

Certain lectins are commonly used in the visualization and identification of blood vessels, as they bind to the carbohydrates of the endothelial cell lining. While useful for staining tissue sections, it is possible to intravenously inject labeled lectins in order to preferentially stain endothelial cells and observe any vascular leakage of the lectin. This can be performed on live animals or on excised tissues. For an excellent example of *Lycopersicon esculentum* (Tomato) lectin (LEL) staining of varied mouse tissues, see Robertson et al., (Ref. 89). Due to differences in the glycans present on endothelial cells among different species, selection of the appropriate lectin for your species of interest is recommended to obtain an optimal signal. For more information on different agents for imaging of vasculature, see Lokmic and Mitchell (Ref. 90).

Aberrant vasculature morphology is a signature of tumors, and represents a barrier for delivery of therapies, both molecular and cellular, to the entire mass (Ref. 91). Park et al., devised a therapeutic strategy to normalize the vasculature through the delivery of antibodies. They demonstrated the compound's regulatory effect on tumor angiogenesis by staining vessels via LEL perfusion in a murine model of glioma (Ref. 29).

Select Published Applications

- *Imaging host vascularization of engrafted tissues (Ref. 5)*
- *Analysis of vascular permeability and visualization of microvessels (Ref. 23)*
- *Quantifying tissue vascularization for tissue engineering (Ref. 33)*
- *Detection and quantification of tumor angiogenesis (Ref. 21)*
- *Determination of drug delivery on vascular organization (Ref. 29)*
- *Vascular endothelial staining standard for methodology comparison (Ref. 27)*

Procedural Overview

Prepare Lectin Solution

- Dissolve/dilute lectin in sterile PBS at 0.5-2 mg/ml.
- Bring solution to room temperature prior to injection.

Injection

- Prepare animal for injection using proper restraints.
- Visualize and prepare injection site, i.e., lateral tail vein.
- Inject intravenous lectin solution (100-200 µl).
- Apply gentle pressure to close injection site .

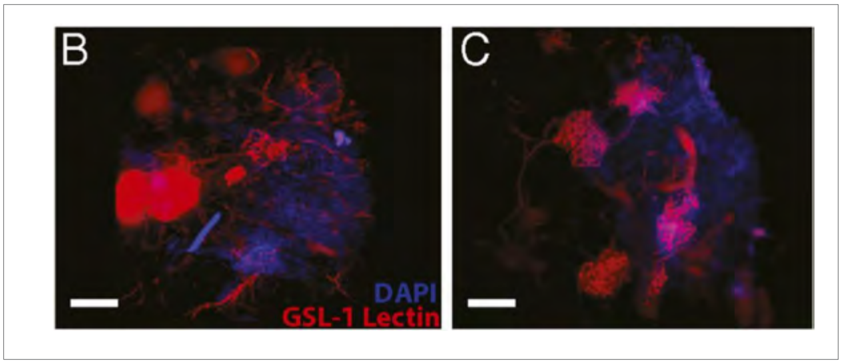
Sacrifice*

- Sacrifice animal(s) 15-30 minutes after injection.

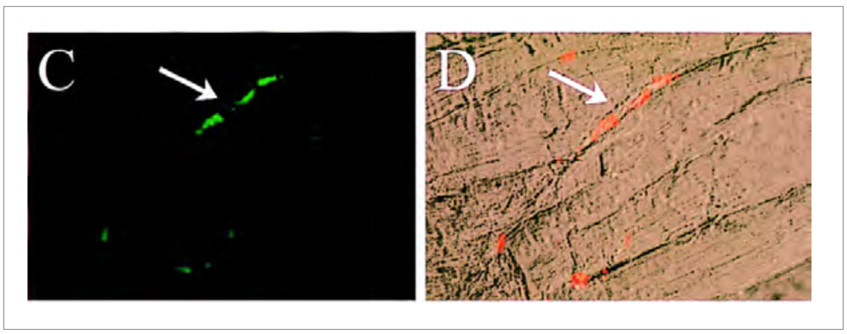
Tissue Preparation and Imaging

- Collect desired tissues for analysis and process for appropriate readout.
- Image capture by desired technique.

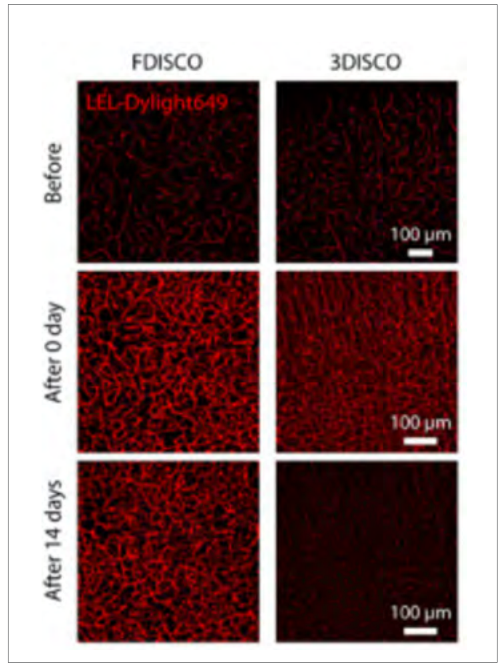
**All animal handling and techniques must be enacted in compliance with IACUC guidelines and with a protocol approved by an institutional animal facility.*



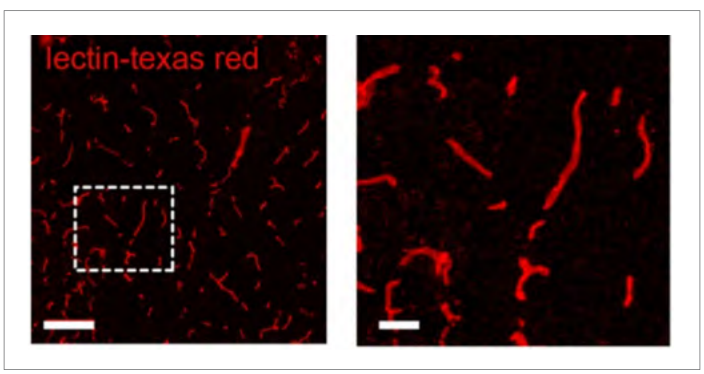
Monitoring vascularization of implanted islet cells embedded within endothelized modules. Unconjugated GSL I (Fig. 3, Ref. 107).



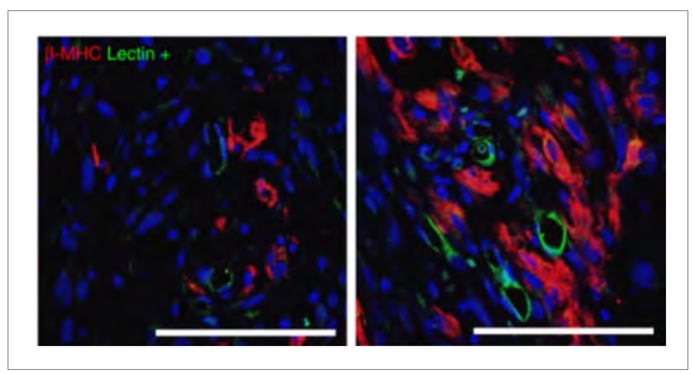
Microscopy reveals incorporation of ex vivo expanded human endothelial progenitor cells into neovascularization sites in ischemic mouse muscle tissue (UEA I green, DiI red). FITC UEA I. (Fig. 4, Ref. 108, © 2000 National Academy of Sciences, U.S.A.).



Comparison of optical clearing methods in mouse brain. DyLight 649 LEL (Fig. 2F, Ref. 27).



Graft vascularization of infarcted rat heart. Texas Red LEL (Fig. 2, Ref. 21).



Graft vascularization of infarcted rat heart. Texas Red LEL (Fig. 7, Ref. 33).

Lectin validation data from the NCFG

The National Center for Functional Glycomics (NCFG), with the support of the NIH Common Fund for Glycosciences, is screening a selection of lectins from Vector Laboratoreis. Using Consortium for Functional Glycomics (CFG) and NCFG glycan arrays, the NCFG analyzes the glycan specificity of each of our lectin lots, and provides these detailed lectin-glycan binding data for each lot on the NCFG website.

The goal of this NCFG project is to support rigorous and reproducible glycobiology experiments.

These data provide uniquely valuable insights into the glycan structures on the glycoproteins being studied:

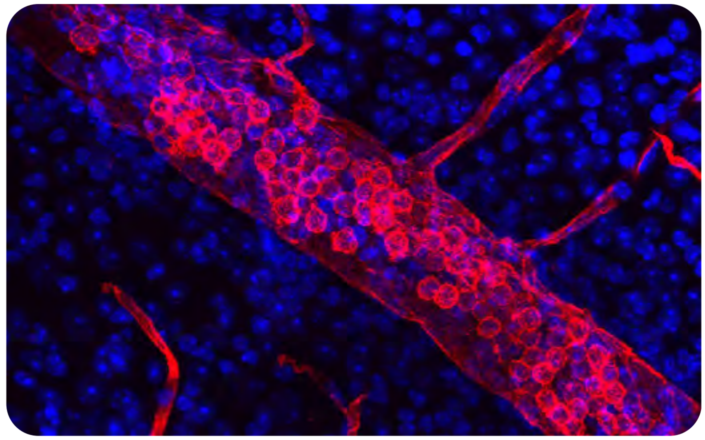
» Binding specificity of each lectin, for each of the hundreds of glycan structures in the array
» Data tabulated and conveniently summarized as a binding array, with peak height indicating degree of binding for each glycan
» Specific and detailed data on complex carbohydrate structure
» Lot-specific data—for confidence in the reproducibility of your binding data

For a more comprehensive review of the application of glycan microarrays, see McQuillan et al., (Ref. 92).

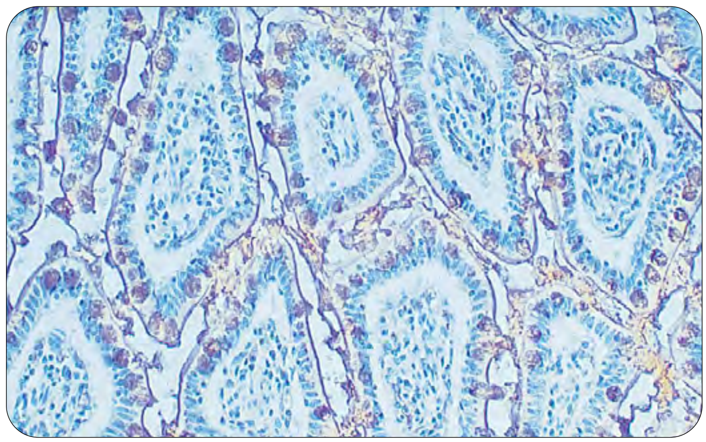
National Center for Functional Glycomics NCFG

Summary of NCFG Services

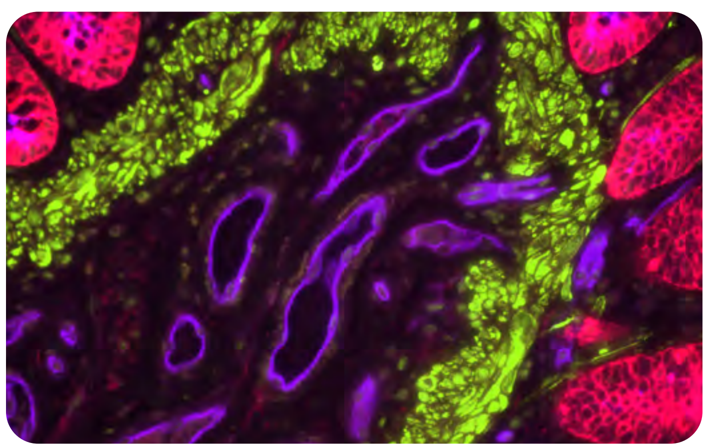
- Analysis of carbohydrate structures from glycoproteins via mass spectrometry
- Determining lectin glycan binding specificity of a sample against:
  » Defined glycan microarrays
  » Naturally derived shotgun glycan microarrays
- Custom array design and printing
- And more! Contact the NCFG to further your glycoscience needs: https://ncfg.hms.harvard.edu



Retina from mice, perfused with DyLight 594-LEL. The retina were sectioned and mounted in VECTASHIELD HardSet with DAPI. Image courtesy of George W. Smith, Florida Atlantic University.



Small intestine: Jacalin (brown, Vector DAB), Vector Hematoxylin counterstain.

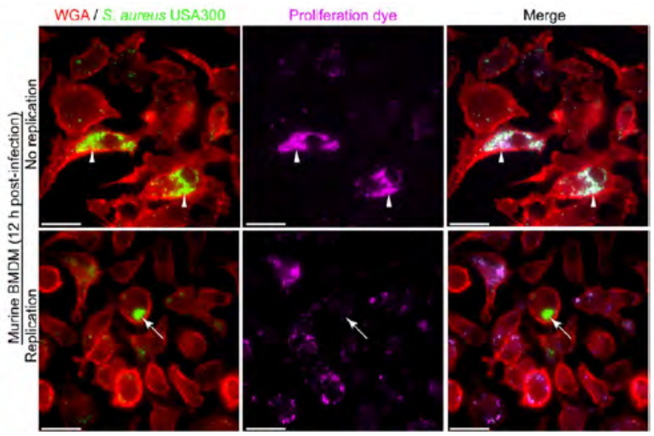


Human Colon: Rabbit Anti-Cytokeratin (AE1/AE3) and Mouse Anti-Desmin detected simultaneously with VectaFluor™ Duet Double Labeling Kit; Vasculature detected using DyLight 649 UEA I Lectin (purple). Mounted in VECTASHIELD PLUS Antifade Mounting Medium.

Other Lectin Applications

Bacterial Assays

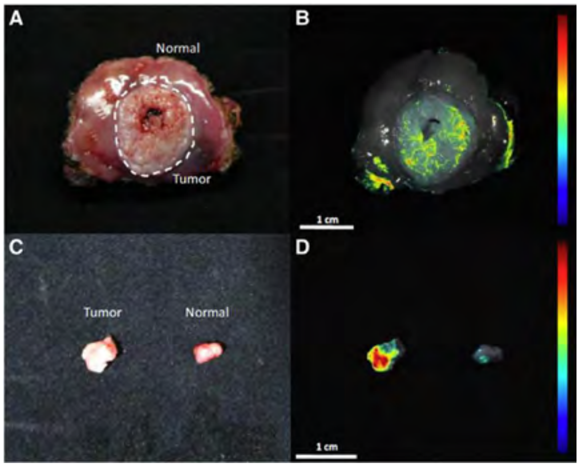
Fluorescent counterstain for intracellular bacterial growth assay.



WGA staining shows cellular localization for intracellular bacteria proliferation assays (Fig. 2, Ref. 41).

Cancer Markers

Multi-spectral epi-illumination to distinguish normal from cancerous.



Directly labeled WGA staining differentiates cancerous versus normal tissue (Fig. 2, Ref. 4).

Lectins Used for Coronavirus Research

Lectins from various sources have been shown to exhibit potent antiviral properties by inhibiting infection of clinically important viral pathogens. The antiviral activity of lectins is largely attributed to direct binding to viral envelope glycans and preventing entry of the virus into cells. Several lectins, particularly plant lectins with affinity toward mannose (Man) and N-acetylglucosamine (GlcNAc) sugar moieties, have been identified as potential therapeutic agents in the prevention of viral transmission in human immunodeficiency virus (HIV) and coronaviruses (SARS-CoV and MERS-CoV) [CVR1]. Promising results in vitro have led to published appeals to consider the anti-viral effects of lectins in combating SARS-CoV [CVR6,7]. As potent inhibitors of viral entry to cells, alga lectin griffithsin has demonstrated anti-viral activity for MERS-CoV [CVR8].

The GlcNAc binding plant lectin Urtica dioica agglutinin (UDA) UDA has been administered in vivo in a murine model of SARS-Cov infection, resulting in 'significant protection from weight loss' and a 'substantial therapeutic effect' [CVR9].

Coronaviruses are enveloped single-stranded RNA viruses that contain at least four structural proteins: the membrane (M), envelope (E), spike (S), and nucleocapsid (N) proteins. The heavily glycosylated S protein mediates virus–cell attachment and fusion. There are 20-30 sites of N-glycosylation on the S protein, depending on the coronavirus [CVR4].

Coronavirus S protein N-glycans mediate the activation of the antiviral innate immune response: coating transmissible gastroenteritis coronavirus (TEGV) particles with ConA prior to cellular exposure reduced interferon (IFN-α) production [CVR4,5]. Dendritic cell-specific ICAM-grabbing non-integrin (DC-SIGN), a mammalian expressed C-type lectin, interacts with the glycans of coronaviruses, and has been shown to mediate viral entry in the case of SARS-CoV. Mannose-binding lectin (MBL) can prevent this interaction (and potentially that of others) by blocking viral binding to DC-SIGN, isolated to a single critical N-glycosylation site in SARS-CoV [CVR4]. MBL interferes with the coronavirus entry process by binding to the high-mannose type N-glycans of SARS-CoV via the S protein, thereby preventing viral attachment to target proteins and the host cell [CVR2, 3]. The importance of lectins in viral defense is also illustrated by MBL deficiency, which has been postulated as a susceptibility factor for SARS-CoV [CVR10].

Vector Laboratories is an established manufacturer of many plant lectins that publications describe as valuable tools in ongoing research to elucidate their potential in suppression of viral activity. Below is a list of mannose-specific and mannose/glucose-specific lectins, available in unconjugated and conjugated formats.

- *Galanthus nivalis* (GNL)
- *Narcissus pseudonarcissus* (NPL)
- Concanavalin A (Con A)
- *Lens culinaris* (LCA)
- *Pisum sativum* (PSA)

Based on prior studies of coronaviruses such as SARS-CoV, MERS-CoV, and TEGV, plant lectins (especially mannose binding lectins) may be used to investigate the following properties of the novel coronavirus SARS-CoV-2, that causes COVID-19: 1) viral glycosylation properties, 2) lectin-based binding inhibition and cellular entry, and 3) novel therapeutic strategies based on glycans and lectins.

Select Coronavirus Research References:

CVR1. Mitchell, C. et al. 2017. Antiviral Lectins: Selective Inhibitors of Viral Entry. 142: 37–54. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5414728/) Antiviral Res.

CVR2. Keyaerts, E. et al. 2007. Plant lectins are potent inhibitors of coronaviruses by interfering with two targets in the viral replication cycle. 75(3):179-87. (https://www.sciencedirect.com/science/article/pii/S0166354207002380?via%3Dihub) Antiviral Res.

CVR3. Ritchie, G., et al. 2010. Identification of N-linked carbohydrates from severe acute respiratory syndrome (SARS) spike glycoprotein. 9(2):257-69. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3412594/) Virology.

CVR4. Fung, S. and Liu, DX. 2018. Post-translational modifications of coronavirus proteins: roles and function. 13(6), 405–430. (https://www.futuremedicine.com/doi/full/10.2217/fvl-2018-0008) Future Virol.

CVR5. Charley B, Lavenant L, Delmas B. 1991. Glycosylation is required for coronavirus TGEV to induce an efficient production of IFN alpha by blood mononuclear cells. 33(4), 435–440. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7169555/) Scand. J. Immunol.

CVR6. Mazalovska M, Kouokam JC. 2018. Lectins as Promising Therapeutics for the Prevention and Treatment of HIV and Other Potential Coinfections. Biomed. 3750646. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5964492/) Biomed. Res. Int.

CVR7. De Clercq E. 2006. Potential antivirals and antiviral strategies against SARS coronavirus infections. 4(2): 291–302. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7105749/) Expert Rev Anti Infect Ther.

CVR8. J. K. Millet, K. Séron, R. N. Labitt et al. 2016. Middle East respiratory syndrome coronavirus infection is inhibited by griffithsin. 133, pp. 1–8. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7113895/) Antiviral Res.

CVR9. Y. Kumaki, M. K. Wandersee, A. J. Smith et al. 2011. Inhibition of severe acute respiratory syndrome coronavirus replication in a lethal SARS-CoV BALB/c mouse model by stinging nettle lectin, Urtica dioica agglutinin. 90, no. 1, pp. 22–32. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3085190/) Antiviral Res.

CVR10. Ip WK, Chan KH, Tso GH et al., 2005. Mannose-binding lecting in severe acute respiratory syndrome coronavirus infection. 191(10):1697-704. (https://www.ncbi.nlm.nih.gov/pubmed/15838797) J Infect Dis.

Table of Lectin Properties

Lectin	Common Name	Source	Glycoprotein	Metal Ions Present	Mitogenic Activity	Blood Group Specificity	Preferred Sugar Specificity	Inhibitor or Eluting Sugar
Aleuria aurantia	AAL	*Aleuria aurantia* mushrooms	No	--	No	Non-specific	Fucα6GlcNAc	L-Fuc
Bauhinia purpurea	BPL, BPA	*Bauhinia purpurea alba* (Camel's Foot Tree) seeds	Yes	No	Yes	A,B,O (-SA)	Galβ3GalNAc	Lactose
Concanavalin A	Con A	*Canavalia ensiformis* (Jack Bean) seeds	No	Ca^{++}, Mn^{++}	Yes	Non-specific	αMan, αGlc	MeαMan+ MeαGlc
Datura stramonium	DSL	*Datura stramonium* (Thorn Apple, Jimson Weed) seeds	Yes	No	Yes	A, B, O	$(GlcNAc)_{2\text{-}4}$	Chitin hydrolysate
Dolichos biflorus	DBA	*Dolicos biflorus* (Horse Gram) seeds	Yes	Ca^{++}, Mn^{++}, Mg^{++}, Zn^{++}	No	A_1>>A_2	αGalNAc	GalNAc
Erythrina cristagalli	ECL, ECA	*Erythrina cristagalli* (Coral Tree) seeds	Yes	Ca^{++}, Mn^{++}, Zn^{++}	Yes	Non-specific	Galβ4GlcNAc	Lactose
Galanthus nivalis	GNL	*Galanthus nivalis* (Snowdrop) bulbs	No	No	No	Rabbit	αMan	MeαMan
Griffonia (Bandeiraea) simplicifolia I	GSL I, BSL I	*Griffonia (Bandeiraea) simplicifolia* seeds	Yes	Ca^{++}, Mn^{++}	No	B>>A_1	αGal, αGalNAc	Gal/GalNAc
Griffonia (Bandeiraea) simplicifolia I Isolectin B_4	GSL I-B_4	*Griffonia (Bandeiraea) simplicifolia* seeds	Yes	Ca^{++}, Mn^{++}	No	B	αGal	Gal or Raffinose
Griffonia (Bandeiraea) simplicifolia II	GSL II, BSL II	*Griffonia (Bandeiraea) simplicifolia* seeds	Yes	Ca^{++}, Mn^{++}	No	A (-SA)>>B (-SA)	α or βGlcNAc	Chitin hydrolysate or GlcNAc
Jacalin	Jacalin	*Artocarpus integrifolia* (Jackfruit) seeds	Yes	No	Yes	O (+SA), T antigen	Galβ3GalNAc	Gal or Melibiose
Lens culinaris	LCA, LcH	*Lens culinaris* (lentil) seeds	No	Ca^{++}, Mn^{++}	Yes	Non-specific	αMan, αGlc	MeαMan+ MeαGlc
Lotus tetragonolobus	LTL	*Lotus tetragonolobus, Tetragonolobus purpurea* (Winged Pea, Asparagus Pea) seeds	Yes	Ca^{++}, Mn^{++}	No	O<A_2	αFuc	L-Fuc
Lycopersicon esculentum	LEL, TL	*Lycopersicon esculentum* (tomato) fruit	Yes	--	No	Non-specific	$(GlcNAc)_{2\text{-}4}$	Chitin hydrolysate
Maackia amurensis I	MAL I, MAL	*Maackia amurensis* seeds	Yes	No	Yes	Non-specific	Galβ4GlcNAc	Lactose
Maackia amurensis II	MAL II, MAH	*Maackia amurensis* seeds	Yes	No	Yes	Non-specific	Neu5Acα3Galβ3GalNAc	Human Glycophorin
Maclura Pomifera	MPL	*Maclura pomifera* (Osage Orange) seeds	No	No	Yes	A, B, O (-SA)	Galβ3GalNAc	Gal

Lectin	Common Name	Source	Glycoprotein	Metal Ions Present	Mitogenic Activity	Blood Group Specificity	Preferred Sugar Specificity	Inhibitor or Eluting Sugar
Narcissus pseudonarcissus	NPL, NPA, DL	*Narcissus pseudonarcissus* (Daffodil) bulbs	No	No	No	Rabbit	αMan	MeαMan
Peanut	PNA	*Arachis hypogaea* peanuts	No	Ca^{++}, Mg^{++}	No	T antigen (M, N)	Galβ3GalNAc	Gal
Phaseolus vulgaris Erythroagglutinin (PHA-E)	PHA-E	*Phaseolus vulgaris* (Red Kidney Bean) seeds	Yes	Ca^{++}, Mn^{++}	Yes	A(-SA)	Galβ4GlcNAcβ2Manα6 (GlcNAcβ4) (GlcNAcβ4Manα3) Manβ4	bovine thyroglobulin, acetic acid
Phaseolus vulgaris Leucoagglutinin (PHA-L)	PHA-L	*Phaseolus vulgaris* (Red Kidney Bean) seeds	Yes	Ca^{++}, Mn^{++}	Yes	-	Galβ4GlcNAcβ6 (GlcNAcβ2Manα3) Manα3	bovine thyroglobulin, acetic acid
Pisum sativum	PSA	*Pisum sativum* (Pea) seeds	Trace	Ca^{++}, Mn^{++}	Yes	Non-specific	αMan, αGlc	MeαMan+ MeαGlc
Ricinus communis I	RCA I, RCA_{120}	*Ricinus communis* (Castor Bean) seeds	Yes	No	No	Non-specific	Gal	Gal or Lactose
Sambucus nigra	SNA, EBL	*Sambucus nigra* (Elderberry) bark	Yes	No	No	Non-specific	Neu5Acα6Gal/GalNAc	Lactose in buffered saline and acetic acid
Solanum tuberosum	STL, PL	*Solanum tuberosum* (potato) tubers	Yes	No	No	Non-specific	$(GlcNAc)_{2-4}$	Chitin hydrolysate
Soybean	SBA	*Glycine max* (soybean) seeds	Yes	Ca^{++}, Mn^{++}	Yes	A>O>B	α>βGalNAc	GalNAc
Ulex europaeus I	UEA I	*Ulex europaeus* (Furze Gorse) seeds	Yes	Ca^{++}, Mn^{++}, Zn^{++}	No	O>A2	αFuc	L-Fuc
Vicia villosa	VVL, VVA	*Vicia villosa* (Hairy Vetch) seeds	Yes	Ca^{++}, Mn^{++}	No	Tn antigen	GalNAc	GalNAc
Wheat Germ	WGA	*Triticum vulgaris* (wheat germ)	No	Ca^{++}	Yes	A,B,O	GlcNAc	Chitin hydrolysate or GlcNAc with acid or salt
Succinylated Wheat Germ	Succinylated WGA	*Triticum vulgaris* (wheat germ)	No	Ca^{++}	No	A,B,O	GlcNAc	Chitin hydrolysate or GlcNAc with acid or salt
Wisteria floribunda	WFA, WFL	*Wisteria floribunda* (Japanese Wisteria) seeds	Yes	--	Yes	Non-specific	GalNAc	GalNAc, acetic acid

Sugar abbreviations:

Fuc	*L-Fucose*
Gal	*D-Galactose*
GalNAc	*N-Acetylgalactosamine*
Glc	*D-Glucose*
GlcNAc	*N-Acetylglucosamine*
Man	*Mannose*
MeαGlc	*α-Methylglucoside*
MeαMan	*α-Methylmannoside*
Neu5Ac	*N-Acetylneuraminic acid (sialic acid)*
SA	*Sialic Acid*

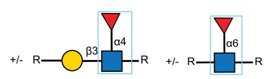
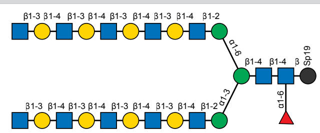
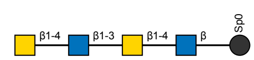
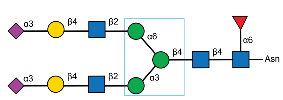
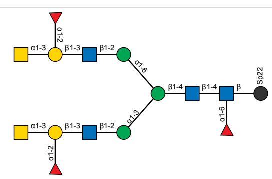
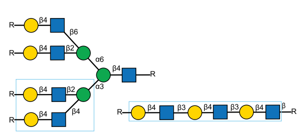
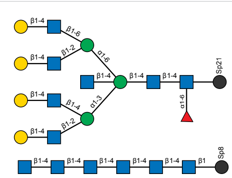
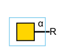
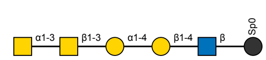
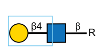
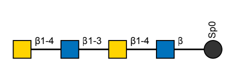
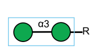
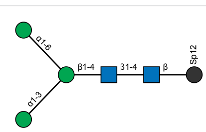
Lectin Properties with Structures

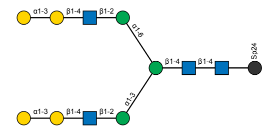
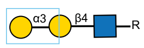
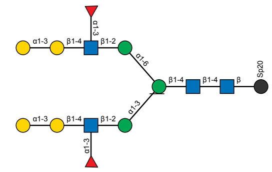
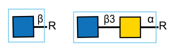
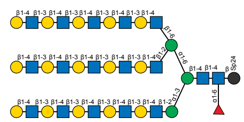
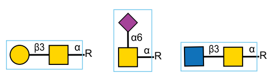
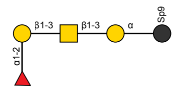
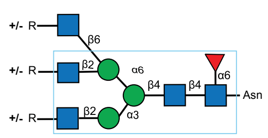
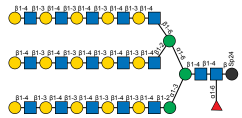
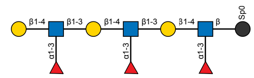
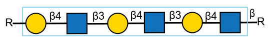
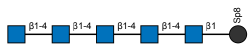
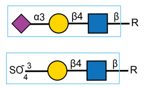
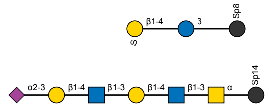
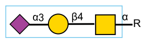
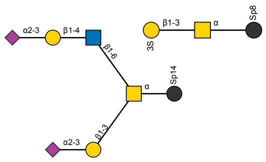
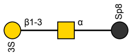
Adapted from Essentials of Glycobiology 3e, with permission from The Consortium of Glycobiology Editors, La Jolla, California; published by Cold Spring Harbor Laboratory Press

For more information: https://cshlpress.org/default.tpl?action=full&src=pdf&--eqskudatarq=1076 and https://www.ncbi.nlm.nih.gov/books/NBK453096/).

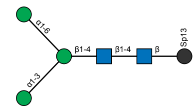
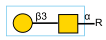
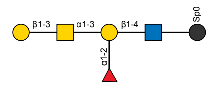
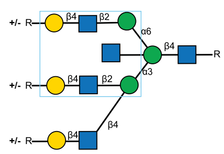
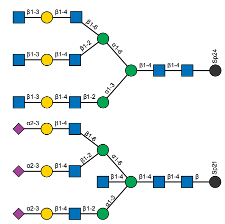
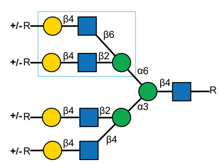
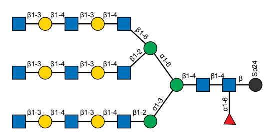
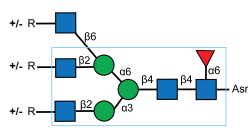
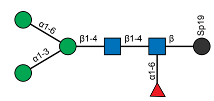
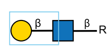
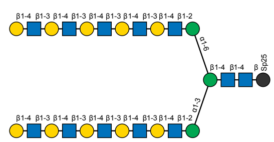
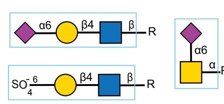
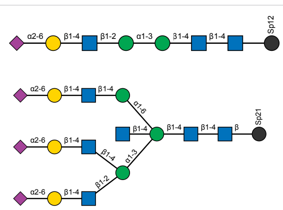
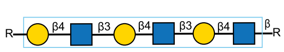
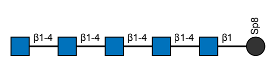
Each lectin is a unique protein that has evolved to interact with distinct carbohydrate epitopes. Deriving from many different species, if grouped by the terminal sugar that the lectin generally binds, each will differ in the strength of binding due to affinity and avidity for the epitope. This may be due to the protein interacting with the rest of the underlying carbohydrate and/or carrier structure (N-glycan, O-glycan, glycolipid, etc.), or due to the presentation and density of the epitope. For example, the glycans may be: tethered to the plasma membrane of a cell, expressed as a recombinant or purified as a recombinant or purified glycoprotein in solution, bound to a solid surface (arrays, beads, plate, etc.), or have a high density of glycans (GAGs). Thus, although we show the primary terminal monosaccharide preferred by each lectin in the following table for ease of use, it does not include the more complex determinants that may exist for each lectin. These subtle, yet distinct, differences in each lectin, and the presentation of their substrates are important to consider when selecting which to use in your application(s) (Refs. 67 and 110).

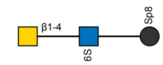
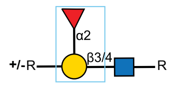
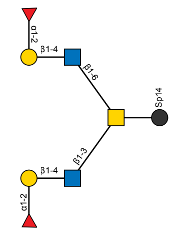
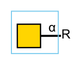
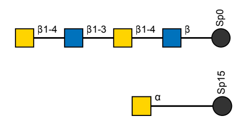
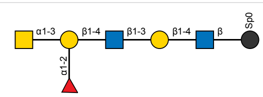
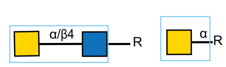
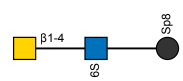
Inhibiting Sugars

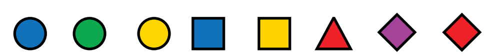
Lectin	Name, Abridged	Inhibitory Sugar[1]	Rough Sugar Specificity[1]	General Binding Motif[2]	Data Source	Top Structure on Array[3]
Aleuria aurantia	AAL	Fuc	Fuc		Data Link	
Bauhinia purpurea	BPL, BPA	Gal	Primanily Gal β-1,3 or 1,4 but will also bind β GalNAc more weakly	—	Data Link	
Concanavalin A	Con A	Man	Branched and terminal mannose [High-Man, Manα-1,6 (Manα-1,3)		Data Link	
Succinylated Concanavalin A	sCon A	Man	αMan, αGlc	—	—	—
Datura stramonium	DSL	Lac	GlcNAc β-1,4 GlcNAc oligomers and LacNAc (Gal β 1,4 GlcNAc)		Data Link	
Dolichos biflorus	DBA	Gal	GlcNAc β-1,4 GlcNAc oligomers and LacNAc (Gal β 1,4 GlcNAc)		Data Link	
Erythrina cristagalli	ECL, ECA	Gal	Gal β-1,4 GalNAc		Data Link	
Galanthus nivalis	GNL	Man	Terminal α-1, 3 mannose		Data Link	

Lectin	Name, Abridged	Inhibitory Sugar[1]	Rough Sugar Specificity[1]	General Binding Motif[2]	Data Source	Top Structure on Array[3]
Griffonia (Bandeiraea) simplicifolia I	GSL I, BSL I	Gal	α-Galactose, also binds some GalNAc	—	Data Link	
Griffonia (Bandeiraea) simplicifolia I Isolectin B_4	GSL I -β_4	Gal	α-Gal		Data Link	
Griffonia (Bandeiraea) simplicifolia II	GSL II, BSL II	GlcNAc	Terminal GlcNAc		Data Link	
Jacalin	Jacalin	Gal	Gal β-1,3 GalNAc		Data Link	
Lens culinaris	LCA, LcH	Man	Complex (man/GlcNAc core with α-1,6 Fuc)		Data Link	
Lotus tetragonolobus	LTL	Fuc	Terminal α-Fuc, Lewis x	—	Data Link	
Lycopersicon esculentum	LEL, TL	GlcNAc	β-1,4 GlcNAc oligomers		Data Link	
Maackia amurensis I	MAL I, MAL	Lac	galactosyl (β-1,4) N-acetylglucosamine, (α-2,3) sialic acid		Data Link	
Maackia amurensis II	MAL II, MAH	Lac	α-2,3 sialic acid-LacNAc structure		Data Link	
Maclura pomutera	MPL	Gal	Gal β-1,3 GalNAc, GalNAc	—	Data Link	

Lectin Properties with Structures (continued)

Lectin	Name, Abridged	Inhibitory Sugar[1]	Rough Sugar Specificity[1]	General Binding Motif[2]	Data Source	Top Structure on Array[3]
Narcissus pseudonarcissus	NPL, NPA, DL	Man	Terminal and internal Man	—	Data Link	
Peanut *(Arachis hyggaea)*	PNA	Gal	Terminal Gal (β-OR)		Data Link	
Phaseolus vulgaris Erythroagglutinin	PHA-E	Lac	Complex-type N-glycans with outer Gal and bisectng GlcNAc		Data Link	
Phaseolus vulgaris Leucoagglutinin	PHA-L	Gal	β-1,6 Brandched trimannosyl core N-linked glycans		Data Link	
Pisum sativum	PSA	Man	Man, (Fuc α-1,6 GlcNAc, α-D-Glc, α-D-Man)		Data Link	
Ricinus communis I	RCA I, RCA_{120}	Lac, Gal	Gal		Data Link	
Sambucus nigra	SNA, EBL	Lac	α-1,6 sialic acid-LacNAc structure		Data Link	
Solanum tuberosum	STL, PL	GlcNAc	GlcNAc oligomers, LacNAc		Data Link	

Lectin	Name, Abridged	Inhibitory Sugar[1]	Rough Sugar Specificity[1]	General Binding Motif[2]	Data Source	Top Structure on Array[3]
Soybean	SBA	Gal	α- or β-Linked terminal GalNAc, GalNAc α-1,3 Gal	—	Data Link	
Ulex europaeus I	UEA I	Fuc	α-Fucose		Data Link	
Vicia villosa	VVL, VVA	GalNAc	GalNAc α, Tn antigen		Data Link	
Wheat Germ	WGA	GlcNAc	β-GlcNAc, sialic acid, GalNAc		Data Link	
Succinylated Wheat Germ	sWGA	GlcNAc	GlcNAc	—	—	—
Wistera floribunda	WFA, WFL	Gal	GalNAc		Data Link	



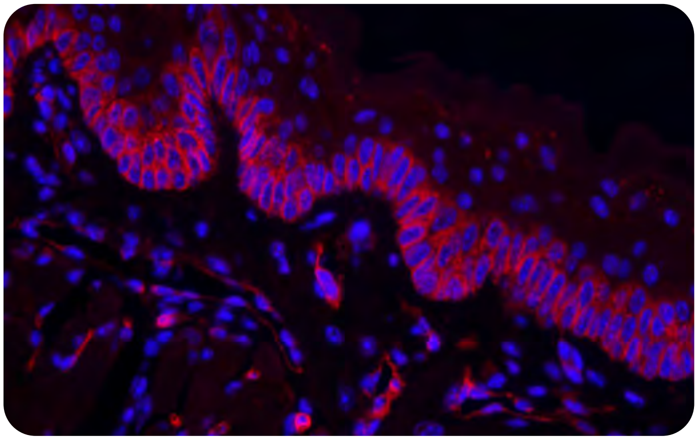
1. Pilobello et al., (95)
2. Essentials of Glycobiology (67)
3. Structures drawn with GLAD (96)
4. Symbol Nomenclature for Glycans (97)

Lectin Products/SKU Table

Lectin	Cat. No.	Unit Size(s)
Aleuria Aurantia Lectin (AAL)		
Unconjugated	L-1390	2 mg
Agarose	AL-1393	2 ml
Biotin	B-1395	1 mg
Fluorescein	FL-1391	1 mg
Bauhinia Purpurea Lectin (BPL)		
Unconjugated	L-1280	5 mg
Concanavalin A (Con A)		
Unconjugated	L-1000	500 mg
Agarose	AL-1003	10 ml, 100 ml
Biotin	B-1005	5 mg
Fluorescein	FL-1001	25 mg
Rhodamine	RL-1002	25 mg
Datura Stramonium Lectin (DSL)		
Unconjugated	L-1180	5 mg
Biotin	B-1185	2 mg
Fluorescein	FL-1181	2 mg
Dolichos Biflorus Agglutinin (DBA)		
Unconjugated	L-1030	5 mg
Biotin	B-1035	5 mg
Fluorescein FL	FL-1031	2 mg, 5 mg
Rhodamine	RL-1032	2 mg
Erythrina Cristagalli Lectin (ECL, ECA)		
Unconjugated	L-1140	10 mg
Agarose	AL-1143	2 ml
Biotin	B-1145	5 mg
Fluorescein	FL-1141	5 mg
Galanthus Nivalis Lectin (GNL)		
Unconjugated	L-1240	5 mg
Agarose	AL-1243	5 ml
Biotin	B-1245	2 mg
Fluorescein	FL-1241	2 mg
Griffonia (Bandeiraea) Simplicifolia Lectin I (GSL I, BSL I)		
Unconjugated	L-1100	5 mg
Biotin	B-1105	2 mg
Fluorescein	FL-1101	2 mg, 5 mg
Rhodamine	RL-1102	2 mg
GSL I – isolectin B_4		
Unconjugated	L-1104	1 mg
Biotin	B-1205	0.5 mg
DyLight 594	DL-1207	0.5 mg
DyLight 649	DL-1208	0.5 mg
Fluorescein	FL-1201	0.5 mg
Griffonia (Bandeiraea) Simplicifolia Lectin II (GSL II, BSL II)		
Unconjugated	L-1210	5 mg
Biotin	B-1215	2 mg
Fluorescein	FL-1211	2 mg
Jacalin		
Unconjugated	L-1150	25 mg
Agarose	AL-1153	10 ml
Biotin	B-1155	5 mg
Fluorescein	FL-1151	5 mg
Lens Culinaris Agglutinin (LCA, LcH)		
Unconjugated	L-1040	10 mg, 25 mg
Biotin	B-1045	5 mg
DyLight™ 649	DL-1048	1 mg
Fluorescein	FL-1041	5 mg
Lotus Tetragonolobus Lectin (LTL)		
Unconjugated	L-1320	5 mg
Biotin	B-1325	2 mg
Fluorescein	FL-1321	2 mg
Lycopersicon Esculentum (Tomato) Lectin (LEL, TL)		
Unconjugated	L-1170	2 mg
Biotin	B-1175	1 mg
DyLight 488	DL-1174	1 mg
DyLight 594	DL-1177	1 mg
DyLight 649	DL-1178	1 mg
Fluorescein	FL-1171	1 mg
Texas Red	TL-1176	1 mg
Maackia Amurensis Lectin I (MAL I, MAL)		
Unconjugated	L-1310	5 mg
Biotin	B-1315	2 mg
Fluorescein	FL-1311	2 mg
Maackia Amurensis Lectin II (MAL II, MAH)		
Unconjugated	L-1260	2 mg
Biotin	B-1265	1 mg
Maclura Pomifera Lectin (MPL)		
Unconjugated	L-1340	5 mg

Narcissus Pseudonarcissus (Daffodil) Lectin (NPL, NPA, DL)		
Unconjugated	L-1370	5 mg
Biotin	B-1375	2 mg
Peanut Agglutinin (PNA)		
Unconjugated	L-1070	5 mg, 25 mg
Agarose	AL-1073	2 ml, 5 ml
Biotin	B-1075	5 mg
CY3	CL-1073	1 mg
CY5	CL-1075	1 mg
Fluorescein	FL-1071	5 mg, 10 mg
Rhodamine	RL-1072	5 mg
Phaseolus Vulgaris Erythroagglutinin (PHA-E)		
Unconjugated	L-1120	5 mg
Biotin	B-1125	2 mg
Fluorescein	FL-1121	2 mg
Phaseolus vulgaris Leucoagglutinin (PHA-L)		
Unconjugated	L-1110	5 mg
Biotin	B-1115	2 mg
Fluorescein	FL-1111	2 mg
Rhodamine	RL-1112	2 mg
Pisum Sativum Agglutinin (PSA)		
Unconjugated	L-1050	10 mg
Biotin	B-1055	5 mg
Ricinus Communis Agglutinin I (RCA I, RCA_{120})		
Unconjugated	L-1080	10 mg
Agarose	AL-1083	2 ml, 10 ml
Biotin	B-1085	5 mg
Fluorescein	FL-1081	5 mg
Rhodamine	RL-1082	5 mg
Sambucus Nigra Lectin (SNA, EBL)		
Unconjugated	L-1300	5 mg
Agarose	AL-1303	2 ml
Biotin	B-1305	2 mg
CY3	CL-1303	1 mg
CY5	CL-1305	1 mg
Fluorescein	FL-1301	2 mg
Solanum Tuberosum (Potato) Lectin (STL, PL)		
Unconjugated	L-1160	5 mg
Biotin	B-1165	2 mg
Soybean Agglutinin (SBA)		
Unconjugated	L-1010	10 mg, 25 mg
Agarose	AL-1013	2 ml
Biotin	B-1015	5 mg
Fluorescein	FL-1011	2 mg

Ulex Europaeus Agglutinin I (UEA I)		
Unconjugated	L-1060	2 mg, 5 mg
Agarose	AL-1063	2 ml
Biotin	B-1065	2 mg
DyLight 594	DL-1067	1 mg
DyLight 649	DL-1068	1 mg
Fluorescein	FL-1061	2 mg, 5 mg
Rhodamine	RL-1062	2 mg
Vicia Villosa Lectin (VVL, VVA)		
Unconjugated	L-1230	5 mg
Agarose	AL-1233	2 ml
Biotin	B-1235	2 mg
Fluorescein	FL-1231	2 mg
Wheat Germ Agglutinin (WGA)		
Unconjugated	L-1020	10 mg, 25 mg
Agarose	AL-1023	2 ml, 5 ml, 10 ml
Biotin	B-1025	5 mg
Fluorescein	FL-1021	5 mg, 10 mg
Peroxidase	PL-1026	2 mg
Rhodamine	RL-1022	5 mg, 10 mg
Succinylated Wheat Germ Agglutinin		
Agarose	AL-1023S	2 ml, 5 ml
Biotin	B-1025S	5 mg
Fluorescein	FL-1021S	5 mg
Wisteria Floribunda Lectin (WFA, WFL)		
Unconjugated	L-1350	5 mg
Agarose	AL-1353	2 ml
Biotin	B-1355	2 mg
Fluorescein	FL-1351	2 mg



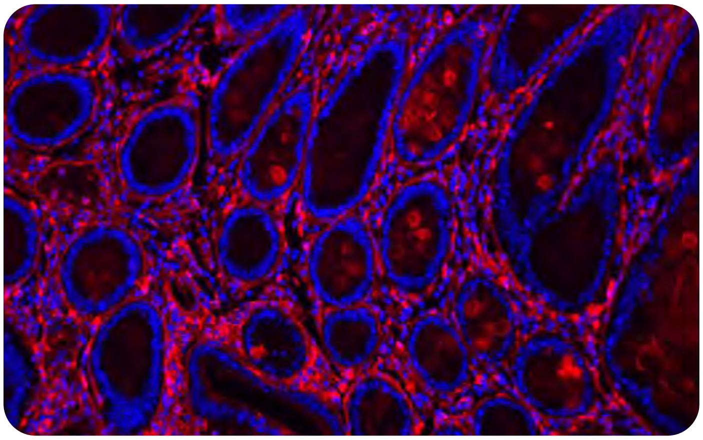
Mouse Tongue: endothelial cells stained with Dylight 594-labeled Griffonia simplicifolia Lectin, Isolectin B4 (red fluorescence). Mounted with VECTASHIELD HardSet with DAPI (blue fluorescence).

Glycan and Lectin Screening Kits

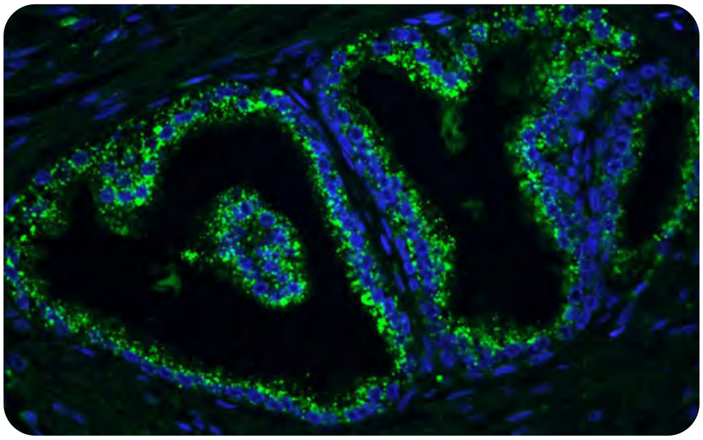
Glycan Screening Kits

Glysite Scout Glycan Screening Kits, Immunofluorescence*	Cat. No.	Unit Size
Glysite™ Scout Glycan Screening Kit, Immunfluorescence 649	GSK-1000	1 kit
Glysite™ Scout Glycan Screening Kit, Immunfluorescence 594	GSK-2000	1 kit
Glysite™ Scout Glycan Screening Kit, Immunfluorescence 488	GSK-3000	1 kit

** The Glysite Scout Glycan Screening Kits, contain the following biotinylated lectins: AAL (Aleuria Aurantia), ECL/ECA (Erythrina Cristagalli), GNL (Galanthus Nivalis), Jacalin, MAL II (Maackia Amurensis II), PHA-L (Phaseolus Vulgaris Leucoagglutinin), WFA/WFL (Wisteria Fluoribunda), and WGA (Wheat Germ Agglutinin). In addition to the glycan binders, the kit includes optimized immunofluorescence reagents for glycan detection, including blocking solutions, streptavidin DyLight™, and VECTASHIELD Vibrance® Antifade Mounting Media with DAPI.*



FFPE human colon carcinoma stained with Glysite Scout Glycan Screen Kit, Immmunofluorescence 594 (red). Nuclear detail was visualized by DAPI staining (blue).



FFPE human prostate stained with Glysite Scout Glycan Screen Kit, Immunofluorescence 488 (green), showing B-GNL staining. Nuclear detail was visualized by DAPI staining (blue).

Lectin Screening Kits

Lectin Screening Kits I	Cat. No.	Unit Size
Biotinylated Lectin Kit I	BK-1000	1 kit
Fluorescein Lectin Kit I	FLK-2100	1 kit
Lectin Screening Kit II		
Biotinylated Lectin Kit II	BK-2100	1 kit
Lectin Screening Kit III		
Biotinylated Lectin Kit III	BK-3000	1 kit

- **Kit I (BK-1000, FLK-2100)** consists of 1 mg of the following lectins or lectin conjugates: Con A, DBA, PNA, RCA I, SBA, UEA I, WGA.
- **Kit II (BK-2100)** consists of 1 mg of the following lectins or lectin conjugates: GSL I, LCA, PHA-E, PHA-L, PSA, Succinylated WGA.
- **Kit III (BK-3000)** consists of 0.5 mg of the following lectin conjugates: DSL, ECL, GSL II, Jacalin, LEL, STL, VVL.

Inhibiting Sugars

Product	Cat. No.	Unit Size	Stock Concentration*
Chitin Hydrolysate	SP-0090	10 ml	N.A.
Sugars			
N-acetylgalactosamine	S-9001	111 mg	100 mM
N-acetylglucosamine	S-9002	442 mg	400 mM
galactose	S-9003	360 mg	400 mM
lactose	S-9004	721 mg	400 mM
α-methylmannoside	S-9005	388 mg	400 mM
α-methylglucoside	S-9006	388 mg	400 mM
L-fucose	S-9007	82 mg	100 mM
N-acetylneuraminic acid (sialic acid)	S-9008	619 mg	400 mM

Antibodies to Lectins

Product	Conjugate	Cat. No.	Unit Size
Anti-*Griffonia (Bandeiraea) Simplicifolia* Lectin I	Unconjugated	AS-2104	1 mg
Anti-Peanut agglutinin	Unconjugated	AS-2074	1 mg
	Biotinylated	BA-0074	0.5 mg
Anti-*Phaseolus vulgaris* agglutinin (E+L)	Unconjugated	AS-2224	1 mg
Anti-*Phaseolus vulgaris* agglutinin (E+L)*	Unconjugated	AS-2300	1 mg
Anti-*Ricinus communis* agglutinin I & II	Unconjugated	AS-2084	1 mg
Anti-Wheat Germ agglutinin	Unconjugated	AS-2024	1 mg

** Stock concentration if reconstituted in 5 ml.*

Glycoprotein Eluting Solutions

Glycoprotein Eluting Solution for Agarose Bound:		
Mannose- or Glucose-binding Lectins	ES-1100	100 ml
Galactose- or GalNAc-binding Lectins	ES-2100	100 ml
GlcNAc- or Chitin-binding Lectins	ES-5100	100 ml
Sialic Acid-binding Lectins	ES-7100	100 ml

Glycoproteins are frequently isolated and purified from protein mixtures using columns of agarose-bound lectins. After applying a protein mixture, the agarose-lectin column is washed free of unwanted proteins and the glycoprotein bound to the lectin is eluted with a sugar that inhibits binding. Unfortunately, achieving complete elution with a simple sugar solution can be difficult. Vector Laboratories has developed five Glycoprotein Elution Solutions in the neutral pH range that maximize the yield of eluted glycoproteins and preserve the activity of the agarose-bound lectins for repeated use. All components of these ready-to-use Glycoprotein Eluting Solutions can subsequently be removed by dialysis.

Agarose-Bound Lectins	Glycoprotein Eluting Solutions			
	ES-1100	ES-2100	ES-5100	ES-7100
AAL (AL-1393)				
Con A (AL-1003)	+			
ECA (AL-1143)		+		
GNL (AL-1243)	+			
Jacalin (AL-1153)		+		
PNA (AL-1073)		+		
RCA_{120} (AL-1083)		+		
SNA (AL-1303)				+
SBA (AL-1013)		+		
UEA I (AL-1063)				
VVA (AL-1233)		+		
WGA (AL-1023)			+	
sWGA (AL-1023S)			+	
WFL (AL-1353)		+		

+ *Indicates recommendation for eluting glycoproteins from agarose-bound lectins.*

Ancillary Products and Reagents

Ancillary Products and Reagents	Catalog Number	Unit Size
ImmEdge® Hydrophobic Barrier Pen	H-4000	2-Pens
Carbo-Free™ Blocking (CFB) Solution (10x Concentration)	SP-5040	125 ml
Vector® TrueVIEW® Autofluorescence Quenching Kit (No Counterstain)	SP-8400	1 Kit
Vector® TrueVIEW® Autofluorescence Quenching Kit (with DAPI Counterstain)	SP-8500	1 Kit
VECTASHIELD® Antifade Mounting Medium (non-hardening formulation)	H-1000	10 ml
VECTASHIELD® Antifade Mounting Medium with DAPI (non-hardening formulation)	H-1200	10 ml
VECTASHIELD Vibrance® Antifade Mounting Medium (hard-setting formulation)	H-1700	2 ml, 10 ml
VECTASHIELD Vibrance® Antifade Mounting Medium with DAPI (hard-setting formulation)	H-1800	2 ml, 10 ml
VECTASHIELD® PLUS Antifade Mounting Medium (non-hardening formulation)	H-1900	2 ml, 10 ml
VECTASHIELD® PLUS Antifade Mounting Medium with DAPI (non-hardening formulation)	H-2000	2 ml, 10 ml

References

1. Antal M, Petko M. Retrograde transport of the lectin Phaseolus vulgaris leucoagglutinin in frog central nervous system. J.Histochem.Cytochem. 1990 Dec;38(12):1913-7
2. Chou TC, Bjorkum AA, Gaus SE, Lu J, Scammell TE, Saper CB. Afferents to the ventrolateral preoptic nucleus. J.Neurosci. 2002 Feb 1;22(3):977-90. PMCID:PMC6758527
3. Srinivasan K, Roy S, Washburn N, Sipsey SF, Meccariello R, Meador JW, III, Ling LE, Manning AM, Kaundinya GV. A Quantitative Microtiter Assay for Sialylated Glycoform Analyses Using Lectin Complexes. J.Biomol.Screen. 2015 Jul;20(6):768-78. PMCID:PMC4512520
4. Jones MB, Oswald DM, Joshi S, Whiteheart SW, Orlando R, Cobb BA. B-cell-independent sialylation of IgG. Proc.Natl.Acad.Sci.U.S.A 2016 Jun 28;113(26):7207-12. PMCID:PMC4932940
5. Watson CL, Mahe MM, Munera J, Howell JC, Sundaram N, Poling HM, Schweitzer JI, Vallance JE, Mayhew CN, Sun Y, et al. An in vivo model of human small intestine using pluripotent stem cells. Nat.Med. 2014 Nov;20(11):1310-4. PMCID:PMC4408376
6. Nawa Y, Teshima T, Sunami K, Hiramatsu Y, Maeda Y, Yano T, Shinagawa K, Ishimaru F, Omoto E, Harada M. G-CSF reduces IFN-gamma and IL-4 production by T cells after allogeneic stimulation by indirectly modulating monocyte function. Bone Marrow Transplant. 2000 May;25(10):1035-40
7. Sofuni T, Yoshida MC. Combined use of several mitogens for mitotic stimulation to human lymphocytes. J.Radiat.Res. 1992 Mar;33 Suppl:222-30
8. Stolfa G, Mondal N, Zhu Y, Yu X, Buffone A, Jr., Neelamegham S. Using CRISPR-Cas9 to quantify the contributions of O-glycans, N-glycans and Glycosphingolipids to human leukocyte-endothelium adhesion. Sci.Rep. 2016 Jul 26;6:30392. PMCID:PMC4960646
9. Pagan JD, Kitaoka M, Anthony RM. Engineered Sialylation of Pathogenic Antibodies In Vivo Attenuates Autoimmune Disease. Cell 2018 Jan 25;172(3):564-77. PMCID:PMC5849077
10. Pauthner M, Havenar-Daughton C, Sok D, Nkolola JP, Bastidas R, Boopathy AV, Carnathan DG, Chandrashekar A, Cirelli KM, Cottrell CA, et al. Elicitation of Robust Tier 2 Neutralizing Antibody Responses in Nonhuman Primates by HIV Envelope Trimer Immunization Using Optimized Approaches. Immunity. 2017 Jun 20;46(6):1073-88. PMCID:PMC5483234
11. Badr HA, AlSadek DM, Mathew MP, Li CZ, Djansugurova LB, Yarema KJ, Ahmed H. Lectin staining and Western blot data showing differential sialylation of nutrient-deprived cancer cells to sialic acid supplementation. Data Brief. 2015 Dec;5:481-8. PMCID:PMC4631887
12. Gutierrez GR, Haseley SR, van Miegem VF, Vliegenthart JF, Kamerling JP. Identification of carbohydrates binding to lectins by using surface plasmon resonance in combination with HPLC profiling. Glycobiology 2004 May;14(5):373-86
13. Wang W, Soriano B, Chen Q. Glycan profiling of proteins using lectin binding by Surface Plasmon Resonance. Anal.Biochem. 2017 Dec 1;538:53-63
14. Jones MB, Nasirikenari M, Lugade AA, Thanavala Y, Lau JT. Anti-inflammatory IgG production requires functional P1 promoter in beta-galactoside alpha2,6-sialyltransferase 1 (ST6Gal-1) gene. J.Biol.Chem. 2012 May 4;287(19):15365-70. PMCID:PMC3346113
15. Mandai M, Ikeda H, Jin ZB, Iseki K, Ishigami C, Takahashi M. Use of lectins to enrich mouse ES-derived retinal progenitor cells for the purpose of transplantation therapy. Cell Transplant. 2010;19(1):9-19
16. Li C, Chen P, Smith MS. Morphological evidence for direct interaction between arcuate nucleus neuropeptide Y (NPY) neurons and gonadotropin-releasing hormone neurons and the possible involvement of NPY Y1 receptors. Endocrinology 1999 Nov;140(11):5382-90
17. Holdbrooks AT, Britain CM, Bellis SL. ST6Gal-I sialyltransferase promotes tumor necrosis factor (TNF)-mediated cancer cell survival via sialylation of the TNF receptor 1 (TNFR1) death receptor. J.Biol.Chem. 2018 Feb 2;293(5):1610-22. PMCID:PMC5798293
18. Dusoswa SA, Horrevorts SK, Ambrosini M, Kalay H, Paauw NJ, Nieuwland R, Pegtel MD, Wurdinger T, Van KY, Garcia-Vallejo JJ. Glycan modification of glioblastoma-derived extracellular vesicles enhances receptor-mediated targeting of dendritic cells. J.Extracell.Vesicles. 2019;8(1):1648995. PMCID:PMC6713149
19. Wang K, Peng ED, Huang AS, Xia D, Vermont SJ, Lentini G, Lebrun M, Wastling JM, Bradley PJ. Identification of Novel O-Linked Glycosylated Toxoplasma Proteins by Vicia villosa Lectin Chromatography. PLoS.One. 2016;11(3):e0150561. PMCID:PMC4780768
20. Giovannone N, Antonopoulos A, Liang J, Geddes SJ, Kudelka MR, King SL, Lee GS, Cummings RD, Dell A, Barthel SR, et al. Human B Cell Differentiation Is Characterized by Progressive Remodeling of O-Linked Glycans. Front Immunol. 2018;9:2857. PMCID:PMC6302748
21. Breckwoldt MO, Bode J, Kurz FT, Hoffmann A, Ochs K, Ott M, Deumelandt K, Kruwel T, Schwarz D, Fischer M, et al. Correlated magnetic resonance imaging and ultramicroscopy (MR-UM) is a tool kit to assess the dynamics of glioma angiogenesis. Elife. 2016 Feb 2;5:e11712. PMCID:PMC4755755
22. Smith LK, Boukhaled GM, Condotta SA, Mazouz S, Guthmiller JJ, Vijay R, Butler NS, Bruneau J, Shoukry NH, Krawczyk CM, et al. Interleukin-10 Directly Inhibits CD8(+) T Cell Function by Enhancing N-Glycan Branching to Decrease Antigen Sensitivity. Immunity. 2018 Feb 20;48(2):299-312. PMCID:PMC5935130
23. Debbage PL, Solder E, Seidl S, Hutzler P, Hugl B, Ofner D, Kreczy A. Intravital lectin perfusion analysis of vascular permeability in human micro- and macro- blood vessels. Histochem.Cell Biol. 2001 Oct;116(4):349-59
24. Syed S, Hakala P, Singh AK, Lapatto HAK, King SJ, Meri S, Jokiranta TS, Haapasalo K. Role of Pneumococcal NanA Neuraminidase Activity in Peripheral Blood. Front Cell Infect.Microbiol. 2019;9:218. PMCID:PMC6608562
25. Linman MJ, Taylor JD, Yu H, Chen X, Cheng Q. Surface plasmon resonance study of protein-carbohydrate interactions using biotinylated sialosides. Anal.Chem. 2008 Jun 1;80(11):4007-13. PMCID:PMC2586005
26. Smith EA, Thomas WD, Kiessling LL, Corn RM. Surface plasmon resonance imaging studies of protein-carbohydrate interactions. J.Am. Chem.Soc. 2003 May 21;125(20):6140-8
27. Qi Y, Yu T, Xu J, Wan P, Ma Y, Zhu J, Li Y, Gong H, Luo Q, Zhu D. FDISCO: Advanced solvent-based clearing method for imaging whole organs. Sci. Adv. 2019 Jan;5(1):eaau8355. PMCID:PMC635775

28. Liang W, Mao S, Sun S, Li M, Li Z, Yu R, Ma T, Gu J, Zhang J, Taniguchi N, et al. Core Fucosylation of the T Cell Receptor Is Required for T Cell Activation. Front Immunol. 2018;9:78. PMCID:PMC5796888

29. Park JS, Kim IK, Han S, Park I, Kim C, Bae J, Oh SJ, Lee S, Kim JH, Woo DC, et al. Normalization of Tumor Vessels by Tie2 Activation and Ang2 Inhibition Enhances Drug Delivery and Produces a Favorable Tumor Microenvironment. Cancer Cell 2016 Dec 12;30(6):953-67

30. Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV. Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science 2008 Apr 18;320(5874):373-6. PMCID:PMC2409116

31. Eriksson P, Lindskog C, Engholm E, Blixt O, Waldenstrom J, Munster V, Lundkvist A, Olsen B, Jourdain E, Ellstrom P. Characterization of avian influenza virus attachment patterns to human and pig tissues. Sci.Rep. 2018 Aug 15;8(1):12215. PMCID:PMC6093914

32. 34. Oswald DM, Sim ES, Baker C, Farhan O, Debanne SM, Morris NJ, Rodriguez BG, Jones MB, Cobb BA. Plasma glycomics predict cardiovascular disease in patients with ART-controlled HIV infections. FASEB J. 2019 Feb;33(2):1852-9. PMCID:PMC6338643

33. Redd MA, Zeinstra N, Qin W, Wei W, Martinson A, Wang Y, Wang RK, Murry CE, Zheng Y. Patterned human microvascular grafts enable rapid vascularization and increase perfusion in infarcted rat hearts. Nat. Commun. 2019 Feb 4;10(1):584. PMCID:PMC6362250

34. Zseli G, Vida B, Szilvasy-Szabo A, Toth M, Lechan RM, Fekete C. Neuronal connections of the central amygdalar nucleus with refeeding-activated brain areas in rats. Brain Struct.Funct. 2018 Jan;223(1):391-414. PMCID:PMC5773374

35. Gonzalez-Cabrera C, Garrido-Charad F, Roth A, Marin GJ. The isthmic nuclei providing parallel feedback connections to the avian tectum have different neurochemical identities: Expression of glutamatergic and cholinergic markers in the chick (Gallus gallus). J.Comp Neurol. 2015 Jun 15;523(9):1341-58

36. Varki N, Anderson D, Herndon JG, Pham T, Gregg CJ, Cheriyan M, Murphy J, Strobert E, Fritz J, Else JG, et al. Heart disease is common in humans and chimpanzees, but is caused by different pathological processes. Evol.Appl. 2009 Feb;2(1):101-12. PMCID:PMC3352420

37. Ge XN, Ha SG, Greenberg YG, Rao A, Bastan I, Blidner AG, Rao SP, Rabinovich GA, Sriramarao P. Regulation of eosinophilia and allergic airway inflammation by the glycan-binding protein galectin-1. Proc.Natl.Acad.Sci.U.S.A 2016 Aug 16;113(33):E4837-E4846. PMCID:PMC4995939

38. Li Y, Fu J, Ling Y, Yago T, McDaniel JM, Song J, Bai X, Kondo Y, Qin Y, Hoover C, et al. Sialylation on O-glycans protects platelets from clearance by liver Kupffer cells. Proc.Natl.Acad.Sci.U.S.A 2017 Aug 1;114(31):8360-5. PMCID:PMC5547648

39. Yang WH, Heithoff DM, Aziz PV, Sperandio M, Nizet V, Mahan MJ, Marth JD. Recurrent infection progressively disables host protection against intestinal inflammation. Science 2017 Dec 22;358(6370). PMCID:PMC5824721

40. Baeten J, Suresh A, Johnson A, Patel K, Kuriakose M, Flynn A, Kademani D. Molecular imaging of oral premalignant and malignant lesions using fluorescently labeled lectins. Transl.Oncol. 2014 Apr;7(2):213-20. PMCID:PMC4101339

41. Flannagan RS, Heinrichs DE. A Fluorescence Based-Proliferation Assay for the Identification of Replicating Bacteria Within Host Cells. Front Microbiol. 2018;9:3084. PMCID:PMC6299164

42. Carter TM, Brooks SA. Detection of aberrant glycosylation in breast cancer using lectin histochemistry. Methods Mol.Med. 2006;120:201-16.

43. Fiorentino MA, Paolicchi FA, Campero CM, Barbeito CG. Lectin binding patterns and immunohistochemical antigen detection in placenta and lungs of Brucella abortus-bovine infected fetuses. Open.Vet.J. 2018;8(1):57-63. PMCID:PMC5918125

44. Irons EE, Lee-Sundlov MM, Zhu Y, Neelamegham S, Hoffmeister KM, Lau JT. B cells suppress medullary granulopoiesis by an extracellular glycosylation-dependent mechanism. Elife. 2019 Aug 13;8. PMCID:PMC6713473

45. Dodla MC, Young A, Venable A, Hasneen K, Rao RR, Machacek DW, Stice SL. Differing lectin binding profiles among human embryonic stem cells and derivatives aid in the isolation of neural progenitor cells. PLoS. One. 2011;6(8):e23266. PMCID:PMC3151296

46. Stanley P, Sundaram S. Rapid assays for lectin toxicity and binding changes that reflect altered glycosylation in mammalian cells. Current Protocols in Chemical Biology. 2014 Jun;6(2):117-133. DOI: 10.1002/9780470559277.ch130206.

47. Cao J, Guo S, Arai K, Lo EH, Ning M. Studying extracellular signaling utilizing a glycoproteomic approach: lectin blot surveys, a first and important step. Methods Mol.Biol. 2013;1013:227-33. PMCID:PMC3985769

48. Wang K, Liu C, Hou Y, Zhou H, Wang X, Mai K, He G. Differential Apoptotic and Mitogenic Effects of Lectins in Zebrafish. Front Endocrinol. (Lausanne) 2019;10:356. PMCID:PMC6560201

49. Liao WY, Fugmann SD. Lectins identify distinct populations of coelomocytes in Strongylocentrotus purpuratus. PLoS.One. 2017;12(11):e0187987. PMCID:PMC5695280

50. Damborsky P, Zamorova M, Katrlik J. Determining the binding affinities of prostate-specific antigen to lectins: SPR and microarray approaches. Proteomics. 2016 Dec;16(24):3096-104

51. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 2006 Aug 4;313(5787):670-3

52. Sharon N, Lis H. History of lectins: from hemagglutinins to biological recognition molecules. Glycobiology 2004 Nov;14(11):53R-62R

53. Panda PK, Mukhopadhyay S, Behera B, Bhol CS, Dey S, Das DN, Sinha N, Bissoyi A, Pramanik K, Maiti TK, et al. Antitumor effect of soybean lectin mediated through reactive oxygen species-dependent pathway. Life Sci. 2014 Aug 28;111(1-2):27-35

54. North SJ, Huang HH, Sundaram S, Jang-Lee J, Etienne AT, Trollope A, Chalabi S, Dell A, Stanley P, Haslam SM. Glycomics profiling of Chinese hamster ovary cell glycosylation mutants reveals N-glycans of a novel size and complexity. J.Biol.Chem. 2010 Feb 19;285(8):5759-75. PMCID:PMC2820803

55. Li YW, Wesselingh SL, Blessing WW. Projections from rabbit caudal medulla to C1 and A5 sympathetic premotor neurons, demonstrated with phaseolus leucoagglutinin and herpes simplex virus. J.Comp Neurol. 1992 Mar 22;317(4):379-95

References (continued)

56. Mikkola M, Toivonen S, Tamminen K, Alfthan K, Tuuri T, Satomaa T, Natunen J, Saarinen J, Tiittanen M, Lampinen M, et al. Lectin from Erythrina cristagalli supports undifferentiated growth and differentiation of human pluripotent stem cells. Stem Cells Dev. 2013 Mar 1;22(5):707-16

57. Thiemann S, Baum LG. Galectins and Immune Responses-Just How Do They Do Those Things They Do? Annu.Rev.Immunol. 2016 May 20;34:243-64

58. Sumner JB, Howell SF. Identification of Hemagglutinin of Jack Bean with Concanavalin A. J.Bacteriol. 1936 Aug;32(2):227-37. PMCID:PMC543784

59. Aub JC, Tieslau C, Lankester A. Reactions of Normal and Tumor Cell Surfaces to Enzymes. I. Wheat-Germ Lipase and Associated Mucopolysaccharides. Proc.Natl.Acad.Sci.U.S.A. 1963 Oct;50:613-9. PMCID:PMC221235

60. Hudgin RL, Pricer WE, Jr., Ashwell G, Stockert RJ, Morell AG. The isolation and properties of a rabbit liver binding protein specific for asialoglycoproteins. J.Biol.Chem. 1974 Sep 10;249(17):5536-43

61. Van Damme EJ. History of plant lectin research. Methods Mol.Biol. 2014;1200:3-13

62. Velkov VV, Medvinsky AB, Sokolov MS, Marchenko AI. Will transgenic plants adversely affect the environment? J.Biosci. 2005 Sep;30(4):515-48

63. Does MP, Houterman PM, Dekker HL, Cornelissen BJ. Processing, targeting, and antifungal activity of stinging nettle agglutinin in transgenic tobacco. Plant Physiol 1999 Jun;120(2):421-32. PMCID:PMC59280

64. Lam SK, Ng TB. Lectins: production and practical applications. Appl. Microbiol.Biotechnol. 2011 Jan;89(1):45-55. PMCID:PMC3016214

65. Keyaerts E, Vijgen L, Pannecouque C, Van DE, Peumans W, Egberink H, Balzarini J, Van RM. Plant lectins are potent inhibitors of coronaviruses by interfering with two targets in the viral replication cycle. Antiviral Res. 2007 Sep;75(3):179-87

66. Swanson MD, Winter HC, Goldstein IJ, Markovitz DM. A lectin isolated from bananas is a potent inhibitor of HIV replication. J.Biol.Chem. 2010 Mar 19;285(12):8646-55. PMCID:PMC2838287

67. Cummings RD, Darvill AG, Etzler ME, et al. Glycan-Recognizing Probes as Tools. 2017. In: Varki A, Cummings RD, Esko JD, et al., editors. Essentials of Glycobiology [Internet]. 3rd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015-2017. Chapter 48. Available from: https://www.ncbi.nlm.nih.gov/books/NBK453096/ doi: 10.1101/glycobiology.3e.048.

68. Dan X, Liu W, Ng TB. Development and Applications of Lectins as Biological Tools in Biomedical Research. Med.Res.Rev. 2016 Mar;36(2):221-47

69. Cohen M, Varki NM, Jankowski MD, Gagneux P. Using unfixed, frozen tissues to study natural mucin distribution. J.Vis.Exp. 2012 Sep 21;(67):e3928. PMCID:PMC3490269

70. Munkley J, Elliott DJ. Hallmarks of glycosylation in cancer. Oncotarget. 2016 Jun 7;7(23):35478-89. PMCID:PMC5085245

71. Poiroux G, Barre A, van Damme EJM, Benoist H, Rouge P. Plant Lectins Targeting O-Glycans at the Cell Surface as Tools for Cancer Diagnosis, Prognosis and Therapy. Int.J.Mol.Sci. 2017 Jun 9;18(6). PMCID:PMC5486055

72. Suzuki O. Glycosylation in lymphoma: Biology and glycotherapy. Pathol. Int. 2019 Aug;69(8):441-9

73. Hoffmeister KM, Josefsson EC, Isaac NA, Clausen H, Hartwig JH, Stossel TP. Glycosylation restores survival of chilled blood platelets. Science 2003 Sep 12;301(5639):1531-4

74. Uematsu J, Koyama A, Takano S, Ura Y, Tanemura M, Kihira S, Yamamoto H, Kawano M, Tsurudome M, O'Brien M, et al. Legume lectins inhibit human parainfluenza virus type 2 infection by interfering with the entry. Viruses. 2012 Jul;4(7):1104-15. PMCID:PMC3407897

75. Mitchell CA, Ramessar K, O'Keefe BR. Antiviral lectins: Selective inhibitors of viral entry. Antiviral Res. 2017 Jun;142:37-54. PMCID:PMC5414728

76. Duverger E, Frison N, Roche AC, Monsigny M. Carbohydrate-lectin interactions assessed by surface plasmon resonance. Biochimie 2003 Jan;85(1-2):167-79

77. Larsson EL, Coutinho A. The role of mitogenic lectins in T-cell triggering. Nature 1979 Jul 19;280(5719):239-41

78. Palacios R. Concanavalin A triggers T lymphocytes by directly interacting with their receptors for activation. J.Immunol. 1982 Jan;128(1):337-42

79. Carvalho EVMM, Oliveira WF, Coelho LCBB, Correia MTS. Lectins as mitosis stimulating factors: Briefly reviewed. Life Sci. 2018 Aug 15;207:152-7

80. Fu LL, Zhou CC, Yao S, Yu JY, Liu B, Bao JK. Plant lectins: targeting programmed cell death pathways as antitumor agents. Int.J.Biochem.Cell Biol. 2011 Oct;43(10):1442-9

81. Hassan MA, Rouf R, Tiralongo E, May TW, Tiralongo J. Mushroom lectins: specificity, structure and bioactivity relevant to human disease. Int.J.Mol.Sci. 2015 Apr 8;16(4):7802-38. PMCID:PMC4425051

82. Kristensson K, Olsson Y. Retrograde axonal transport of protein. Brain Res. 1971 Jun 18;29(2):363-5

83. Saleeba C, Dempsey B, Le S, Goodchild A, McMullan S. A Student's Guide to Neural Circuit Tracing. Front Neurosci. 2019;13:897. PMCID:PMC6718611

84. Kobbert C, Apps R, Bechmann I, Lanciego JL, Mey J, Thanos S. Current concepts in neuroanatomical tracing. Prog.Neurobiol. 2000 Nov;62(4):327-51

85. Levy SL, White JJ, Lackey EP, Schwartz L, Sillitoe RV. WGA-Alexa Conjugates for Axonal Tracing. Curr.Protoc.Neurosci. 2017 Apr 10;79:1. PMCID:PMC5830103

86. Goshgarian HG, Buttry JL. The pattern and extent of retrograde transsynaptic transport of WGA-Alexa 488 in the phrenic motor system is dependent upon the site of application. J.Neurosci.Methods 2014 Jan 30;222:156-64. PMCID:PMC4068738

87. Huang Q, Zhou D, DiFiglia M. Neurobiotin, a useful neuroanatomical tracer for in vivo anterograde, retrograde and transneuronal tract-tracing and for in vitro labeling of neurons. J.Neurosci.Methods 1992 Jan;41(1):31-43

88. Vercelli A, Repici M, Garbossa D, Grimaldi A. Recent techniques for tracing pathways in the central nervous system of developing and adult mammals. Brain Res.Bull. 2000 Jan 1;51(1):11-28

89. Robertson RT, Levine ST, Haynes SM, Gutierrez P, Baratta JL, Tan Z, Longmuir KJ. Use of labeled tomato lectin for imaging vasculature structures. Histochem.Cell Biol. 2015 Feb;143(2):225-34

90. Lokmic Z, Mitchell GM. Visualisation and stereological assessment of blood and lymphatic vessels. Histol.Histopathol. 2011 Jun;26(6):781-96

91. Schaaf MB, Garg AD, Agostinis P. Defining the role of the tumor vasculature in antitumor immunity and immunotherapy. Cell Death.Dis. 2018 Jan 25;9(2):115. PMCID:PMC5833710

92. McQuillan AM, Byrd-Leotis L, Heimburg-Molinaro J, Cummings RD. Natural and Synthetic Sialylated Glycan Microarrays and Their Applications. Front Mol.Biosci. 2019;6:88. PMCID:PMC6753469

93. Cummings RD, Etzler ME. Antibodies and Lectins in Glycan Analysis. 2009;

94. Cummings RD, Darvill AG, Etzler ME, Hahn MG. Glycan-Recognizing Probes as Tools. 2015;611-25

95. Pilobello KT, Agrawal P, Rouse R, Mahal LK. Advances in lectin microarray technology: optimized protocols for piezoelectric print conditions. Curr.Protoc.Chem.Biol. 2013;5(1):1-23. PMCID:PMC4734107

96. Mehta AY, Cummings RD. GLAD: GLycan Array Dashboard, a visual analytics tool for glycan microarrays. Bioinformatics. 2019 Sep 15;35(18):3536-7. PMCID:PMC6748710

97. Varki A, Cummings RD, Aebi M, Packer NH, Seeberger PH, Esko JD, Stanley P, Hart G, Darvill A, Kinoshita T, et al. Symbol Nomenclature for Graphical Representations of Glycans. Glycobiology 2015 Dec;25(12):1323-4. PMCID:PMC4643639

98. Hautala LC, Pang P-C, Antonopoulos A, Pasanen A, Lee C-L, Chiu P CN, Yeung WSB, Loukovaara M, Bützow R, Haslam SM, Dell A, Koistinen H. Altered glycosylation of glycodelin in endometrial carcinoma. Lab Invest. 2020 Mar 23. https://doi.org/10.1038/s41374-020-0411-x

99. Ana M. Dias, Alexandra Correia, Márcia S. Pereira, Catarina R. Almeida, Inês Alves, Vanda Pinto, Telmo A. Catarino, Nuno Mendes, Magdalena Leander, M. Teresa Oliva-Teles, Luís Maia, Cristina Delerue-Matos, Naoyuki Taniguchi, Margarida Lima, Isabel Pedroto, Ricardo Marcos-Pinto, Paula Lago, Celso A. Reis, Manuel Vilanova, and Salomé S. Pinho. Metabolic control of T cell immune response through glycans in inflammatory bowel disease. PNAS May 15, 2018 115 (20) E4651-E4660; first published May 2, 2018 https://doi.org/10.1073/pnas.1720409115.

100. Ying-Chih Liu, Hsin-Yung Yen, Chien-Yu Chen, Chein-Hung Chen, Ping-Fu Cheng, Yi-Hsiu Juan, Chung-Hsuan Chen, Kay-Hooi Khoo, Chong-Jen Yu, Pan-Chyr Yang, Tsui-Ling Hsu, and Chi-Huey Wong. Sialylation and fucosylation of epidermal growth factor receptor suppress its dimerization and activation in lung cancer cells. PNAS July 12, 2011 108 (28) 11332-11337; https://doi.org/10.1073/pnas.1107385108.

101. Sweeney, J.G., Liang, J., Antonopoulos, A. et al. Loss of GCNT2/I-branched glycans enhances melanoma growth and survival. Nat Commun 9, 3368 (2018).

102. Peiris D, Spector AF, Lomax-Browne H, Azimi T, Ramesh B, Loizidou M, Welch H, Dwek MV. Cellular glycosylation affects Herceptin binding and sensitivity of breast cancer cells to doxorubicin and growth factors. Scientific Reports volume 7, Article number: 43006 (2017).

103. Panzer SE, Wilson ND, Verhoven BM, Xiang D, Rubinstein CD, Redfield RR, Zhong W, Reese SR. Complete B Cell Deficiency Reduces Allograft Inflammation and Intragraft Macrophages in a Rat Kidney Transplant Model. Transplantation. 2018 Mar;102(3):396-405.

104. Unal G, Joshi A, Viney TJ, Kis V, Somogyi P. Synaptic Targets of Medial Septal Projections in the Hippocampus and Extrahippocampal Cortices of the Mouse. Journal of Neuroscience 2 December 2015, 35 (48) 15812-15826.

105. Richard H. Thompson and Larry W. Swanson. Hypothesis-driven structural connectivity analysis supports network over hierarchical model of brain architecture. PNAS August 24, 2010 107 (34) 15235-15239; https://www.pnas.org/content/107/34/15235

106. Grasielle C. Kincheski,Sandra R. Mota-Ortiz,Eloisa Pavesi,Newton S. Canteras,Antônio P. Carobrez. The Dorsolateral Periaqueductal Gray and Its Role in Mediating Fear Learning to Life Threatening Events. PLOS ONE Published: November 28, 2012.

107. Alexander E. Vlahos, Nicholas Cober, and Michael V. Sefton. Modular tissue engineering for the vascularization of subcutaneously transplanted pancreatic islets. PNAS first published August 16, 2017 https://doi.org/10.1073/pnas.1619216114.

108. Christoph Kalka, Haruchika Masuda, Tomono Takahashi, Wiltrud M. Kalka-Moll, Marcy Silver, Marianne Kearney, Tong Li, Jeffrey M. Isner, and Takayuki Asahara. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. PNAS March 28, 2000 97 (7) 3422-3427; https://doi.org/10.1073/pnas.97.7.3422.

109. Yinghui Song, Vivek Kumar, Hua-Xing Wei, Ju Qiu, and Pamela Stanley. Lunatic, Manic and Radical Fringe Each Promote T and B Cell Development. J Immunol. 2016 Jan 1;196(1):232-43. doi: 10.4049/jimmunol.1402421. Epub 2015 Nov 25.

110. Cummings RD, Etzler ME. Antibodies and Lectins in Glycan Analysis. In: Varki A, Cummings RD, Esko JD, et al., editors. Essentials of Glycobiology. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 45. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1919/

111. Alice Dobi, Elyssa B. Margolis, Hui-Ling Wang, Brandon K. Harvey and Marisela Morales. Glutamatergic and Nonglutamatergic Neurons of the Ventral Tegmental Area Establish Local Synaptic Contacts with Dopaminergic and Nondopaminergic Neurons. Journal of Neuroscience 6 January 2010, 30 (1) 218-229; DOI: https://doi.org/10.1523/JNEUROSCI.3884-09.2010.

Contact Details

Ordering Information

Order online at **vectorlabs.com**

Orders may also be placed by email, telephone, or mail. Please include the following with each order:

- Product name and catalog number
- Unit size and quantity
- Billing and shipping addresses
- Purchase order number
- Name, phone number, address, and email address of person placing order

Orders using VISA, Mastercard, or American Express are accepted and processed immediately. Telephone orders over $1,000 may require written confirmation. A confirmation should be boldly marked “Confirming Order. Do Not Duplicate”. Duplicate shipments due to incorrectly marked confirming orders cannot be returned for credit. No returned product will be accepted or credited without prior authorization from Vector Laboratories.

Please contact us to discuss discounts for custom or large orders.

Payment/shipping terms

For non-credit card orders, our payment terms are net 30 days from date of invoice, title and risk of loss transfer Ex Works (Incoterms 2010) Seller’s location, freight prepaid and added unless shipped on Buyer’s account (FedEx, UPS, DHL). Buyers are required to submit a credit application before credit terms are extended. Orders placed before 3 pm Pacific Time on Monday through Friday (excluding holidays) are usually processed the same day they are received. Unless requested otherwise, all products are shipped 2nd Day Air.

US Office

USA Headquarters:
Vector Laboratories, Inc.
6737 Mowry Avenue
Newark, CA 94560
Tel: +1 (650) 697-3600
Ordering and Technical Service (USA): +1 (800) 227-6666
Customer Service: vector@vectorlabs.com
Technical Support: technical@vectorlabs.com
International Inquiries: techintl@vectorlabs.com

For more information please visit

vectorlabs.com

LIT3003.Rev00

vector LABORATORIES

vectorlabs.com

Together we breakthrough™

Immunohistochemistry • Immunofluorescence
Lectins & Glycobiology • Bioconjugation