Lectins Application and Resource Guide

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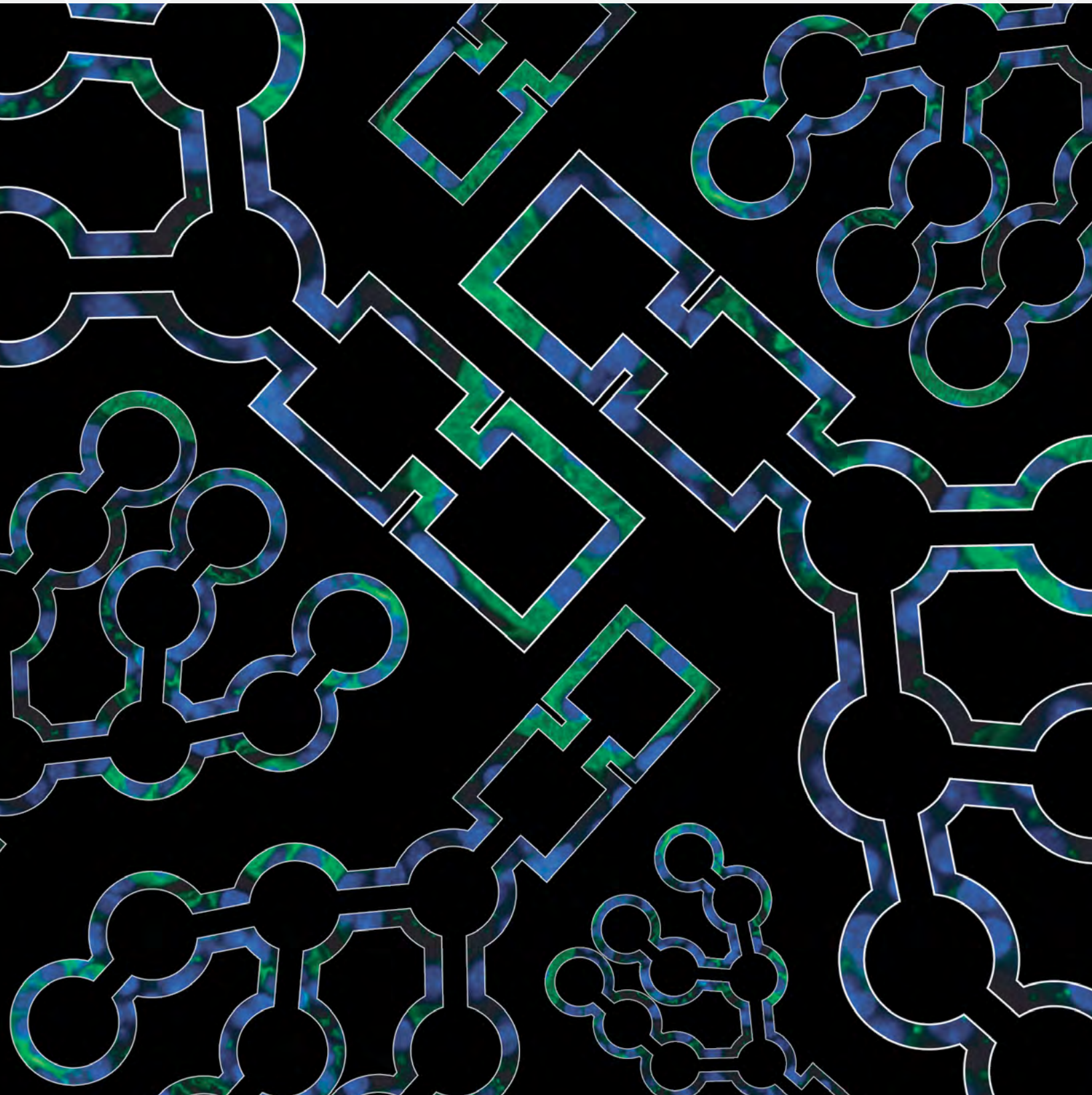

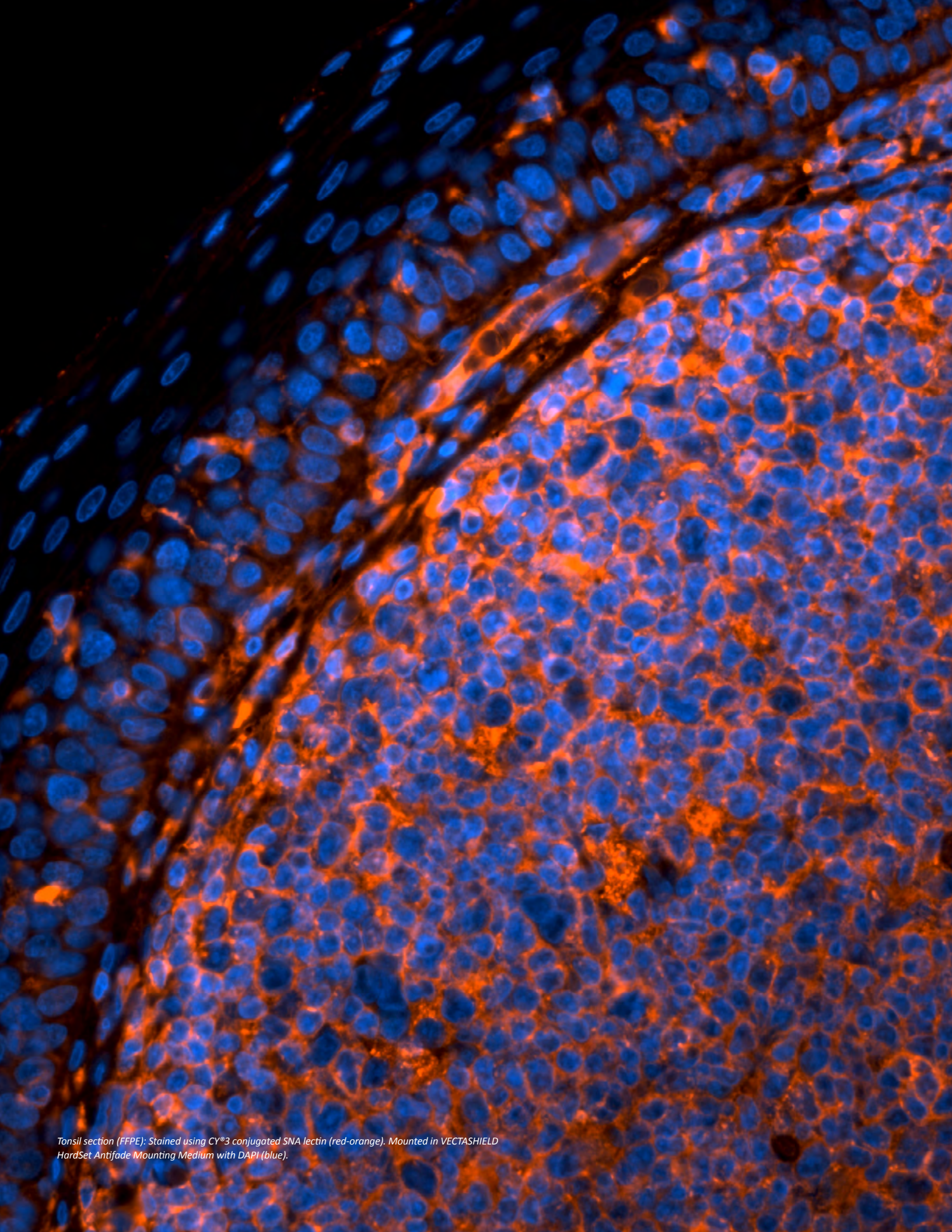

Tonsil section (FFPE): Stained using CY®3 conjugated SNA lectin (red-orange). Mounted in VECTASHIELD HardSet Antifade Mounting Medium with DAPI (blue).

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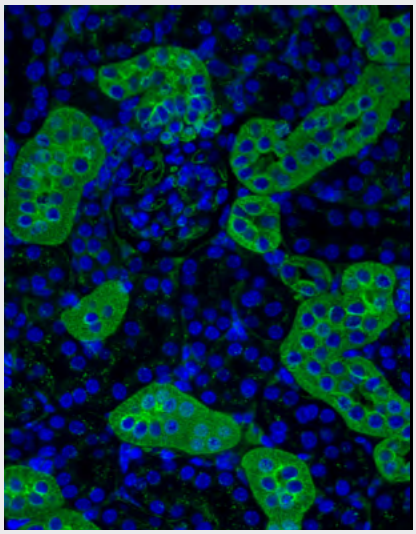
Lectins Application and Resource Guide

Vector Laboratories was founded on a growing portfolio of purified lectins and lectin conjugates that helped to pioneer lectin histochemistry. These products remain a key component of our business today. In the early 1980s, we leveraged our expertise in histochemistry to revolutionize the field of IHC with the commercialization of antibody-based avidin-biotin reagents and the introduction of the VECTASTAIN® ABC system. This system enabled routine immunohistochemistry with any standard brightfield microscope. Following the success of the ABC kits, Vector Laboratories continued to introduce novel and innovative products to support cell and tissue antigen visualization. These include the ImmPRESS® micropolymer reagents, M.O.M.® (Mouse on Mouse) detection systems, unique ImmPACT® enzyme substrates, VECTASHIELD® Antifade Mounting Media and TrueVIEW® Autofluorescence Quenching Kits for immunofluorescence applications. In early 2020, we expanded our bioconjugation portfolio with the addition of SoluLINK® products and services that include a range of conjugation kits, conjugation linkers, magnetic beads & agarose, and biotin & digoxigenin labeling reagents.



Cover: Mouse kidney formalin-fixed, paraffin-embedded: DyLight® 488 conjugated Lycopersicon esculentum lectin (green) mounted in VECTASHIELD HardSet™ Antifade Mounting Medium with DAPI.

*Special thanks and acknowledgement to **Mark B. Jones, PhD** for his work and collaboration on this Lectins Application and Resource Guide.*

Lectins Introduction and Overview

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 Specificity and Glycan Structures 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).

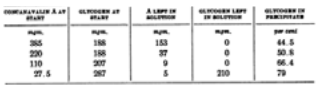
Lectins Timeline

1888
Peter Hermann Stillmark describes lectins.

1890s
Paul Ehrlich shows immune neutralization of toxic lectins.

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

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 a promising agent 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).

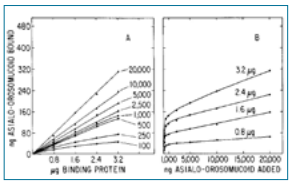
Altered glycosylation is a property of cancer cells, and many plant lectins have shown promising anticancer effects on cancer cells *in vitro* (Ref. 64). Plant lectins have also been shown to have antiviral properties, suppressing the growth and preventing attachment of virions of coronavirus and HIV (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
Irvin E. Liener describes the role of plant lectins as protection from insect seed predators.

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

2004
"...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." — 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 if using FFPE

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

Block

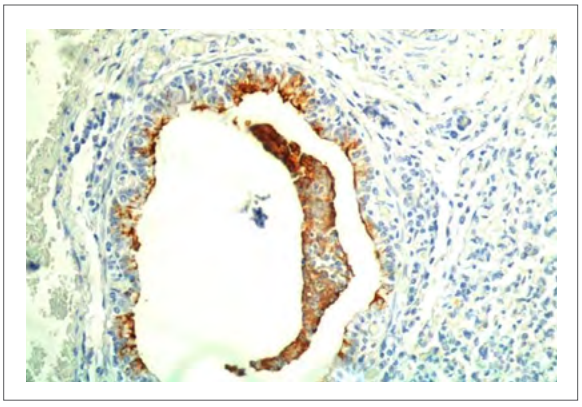
- Incubate slides for 30 minutes at room temperature in Carbo-Free® Blocking (CFB) Solution.

Detection

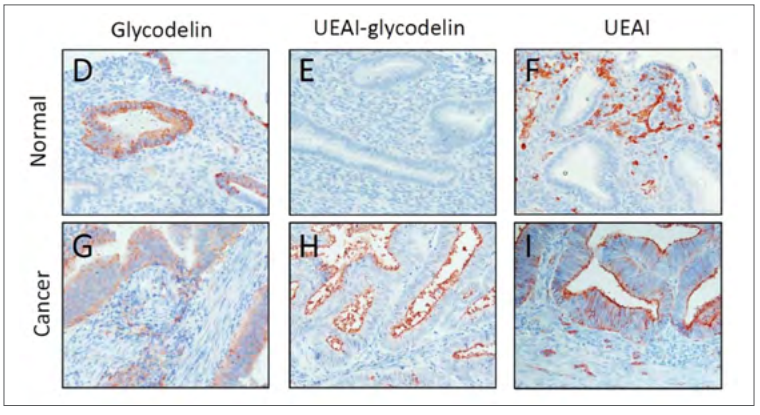
- Incubate slide with biotinylated lectin for 1 hour at room temperature, in CFB Solution.
  Suggested initial working range is 0.5 – 10 µg/mL, user optimization required.
  » *Alternatively, probe overnight at 4°C*
- Wash slide with TBS with gentle agitation for 5 minutes — **perform three times (x3).**
- 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).



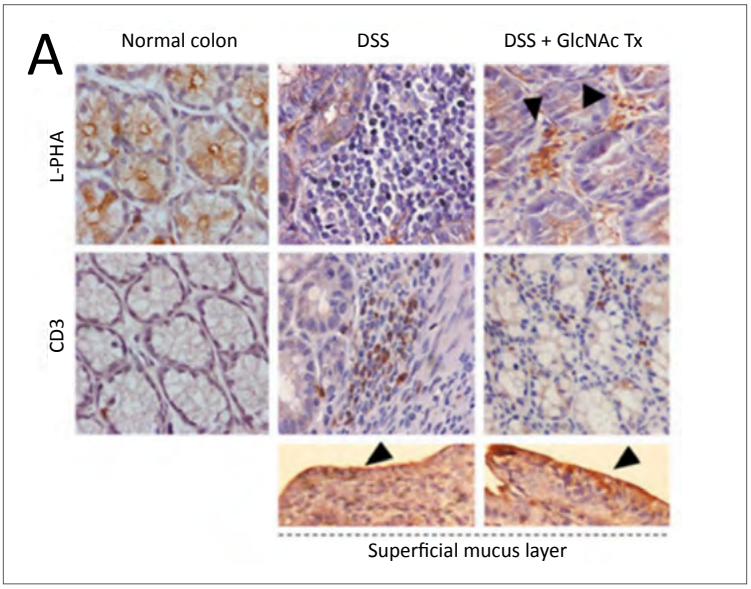
In situ proximity ligation staining utilizing a lectin for detection of endometrial carcinoma. Biotinylated UEA I (Fig. 3 - Ref. 98).

General Immunohistochemistry Workflow

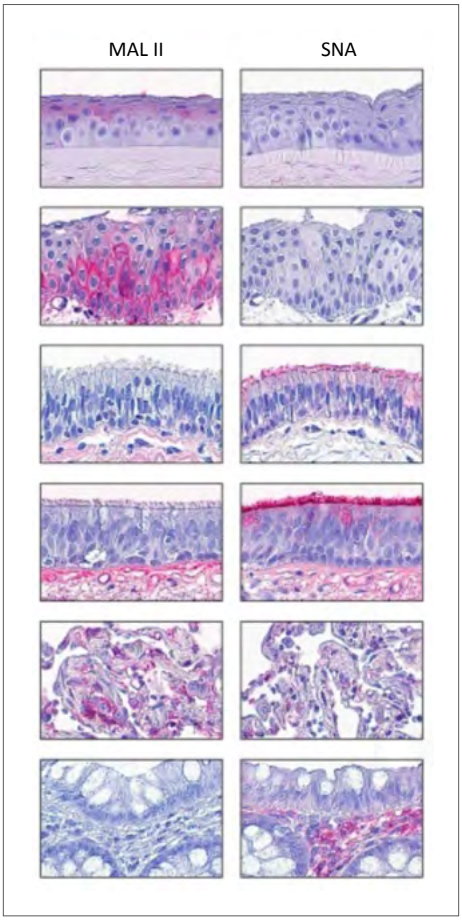
Depending on the intended detection and visualization methodology, various reagent options are available throughout the workflow.

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® Block	• 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 Reagents (HRP) • VECTASTAIN Elite® ABC Reagents (HRP) • VECTASTAIN ABC-AP Reagents (AP) • VECTASTAIN Elite ABC PLUS Kit (HRP) • ImmPRESS Excel Amplified Staining Kits (HRP) • 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® Mounting Medium • VectaMount AQ Mounting Medium	

* For more information visit: vectorlabs.com/browse/lectins



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 8 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 if using FFPE

- 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 µg/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).**

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.

Quench Autofluorescence

- Apply TrueVIEW® Autofluorescence Quenching Kit if autofluorescence obscures specific signal.

Mount/Coverslip

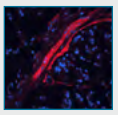
- Apply coverslip with VECTASHIELD Antifade Mounting Media.

Signal Acquisition

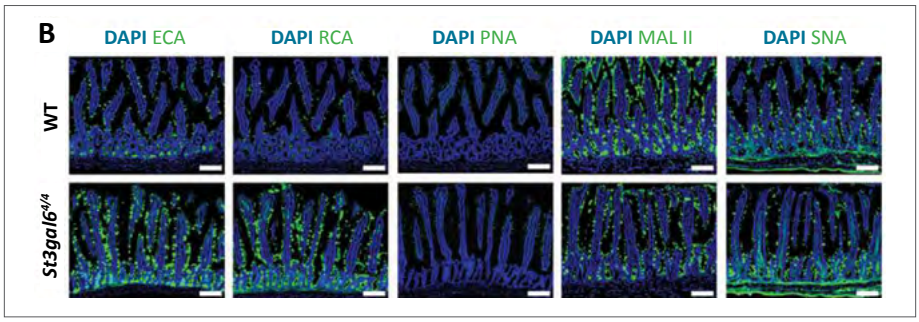
- Image capture via fluorescent microscopy.

General Immunofluorescence Workflow

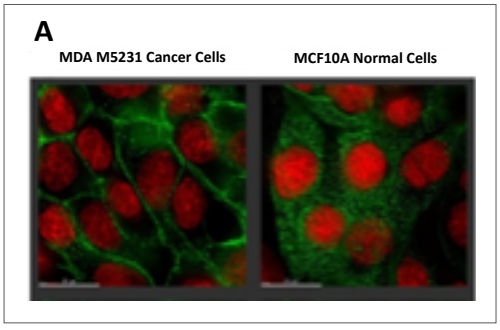
Depending on the intended detection and visualization methodology, various reagent options are available throughout the workflow.

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	• TrueVIEW Autofluorescence Quenching Kit** • TrueVIEW Autofluorescence Quenching Kit with 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 Block • 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	• VECTASHIELDR Antifade Mounting Medium • VECTASHIELD Antifade Mounting Medium with DAPI • VECTASHIELD Antifade Mounting Medium with Propidium Iodide (PI) • VECTASHIELD HardSet Antifade Mounting Medium • VECTASHIELD HardSet Antifade Mounting Medium with DAPI • VECTASHIELD HardSet Antifade Mounting Medium with TRITC-Phalloidin • VECTASHIELD Vibrance™ Antifade Mounting Medium • VECTASHIELD Vibrance Antifade Mounting Medium with DAPI	

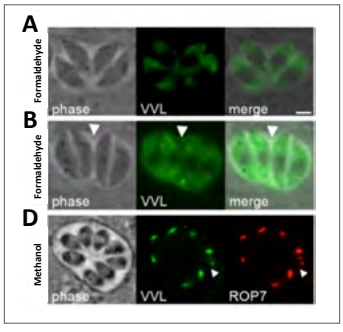
* For more information visit: vectorlabs.com/browse/lectins
** TrueVIEW Autofluorescence Quenching is applied just prior to coverslipping



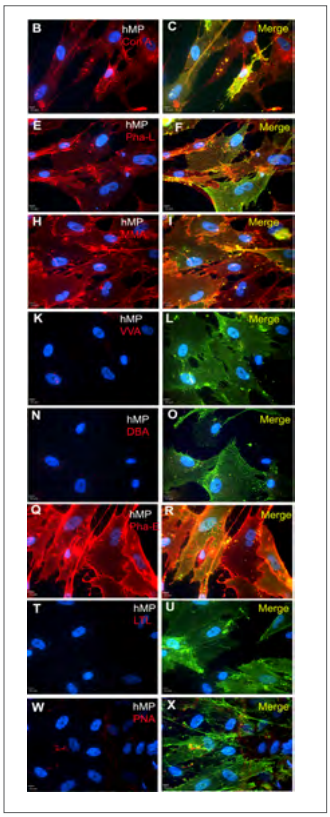
Glycosylation of murine small intestine. Biotinylated ECL/ECA; Biotinylated RCA; Biotinylated PNA; Biotinylated MAL II; Biotinylated SNA (Fig. S5 - Ref. 39).



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



Vicia Villosa Lectin staining in Toxoplasma. FITC VVL (Fig. 1 - 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)

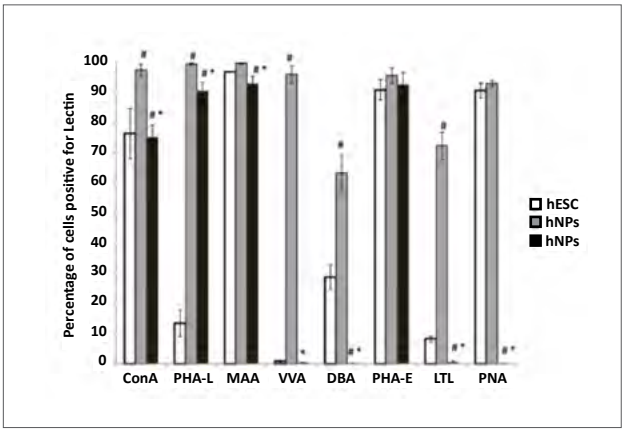
Flow Cytometry – Cell Analysis

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. 45)
- 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

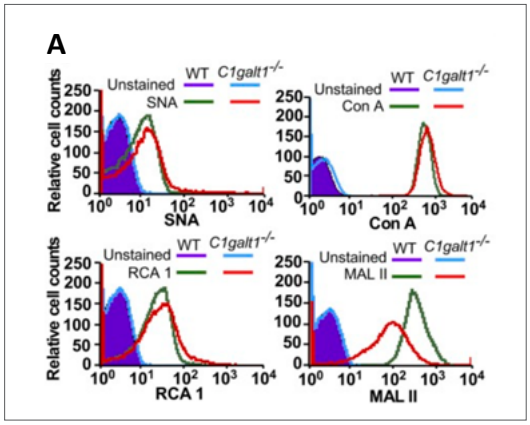
- Incubate cells with fluorophore-conjugated lectin for 1 hour at 4°C, in buffer. Suggested initial working range is 0.5 – 10 µg/mL, user optimization required. Biotinylated lectins are also widely used for flow cytometry applications. See accompanying references and figure images.
- Wash cells with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

Visualization

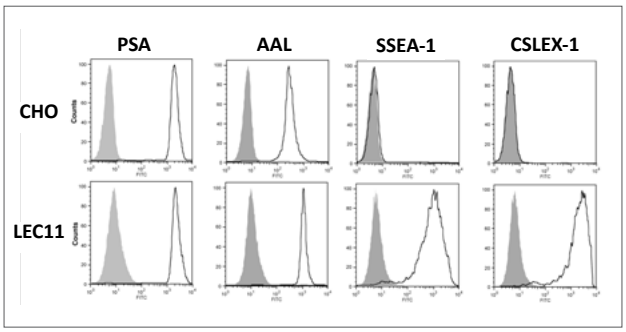
- If using a biotinylated lectin, incubate cells with streptavidin conjugated fluorophore at this step for 1 hour at 4C in CFB Solution.
- Wash cells with cold PBS – **perform two times (x2).**
- Resuspend in PBS at desired concentration.

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).



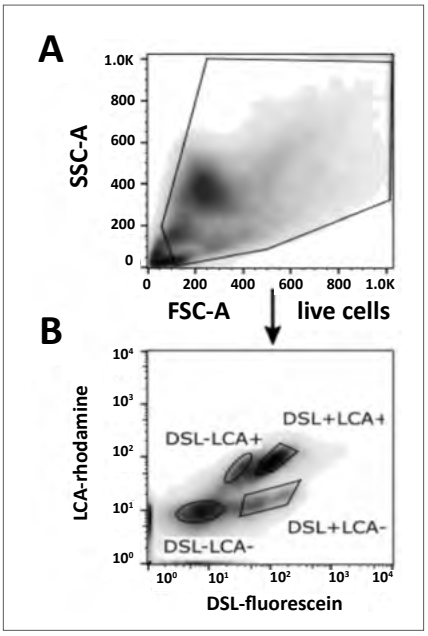
Characterization of CHO cell surface glycosylation. Biotinylated PSA; Biotinylated AAL (Ref. 54).

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 sub-populations 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

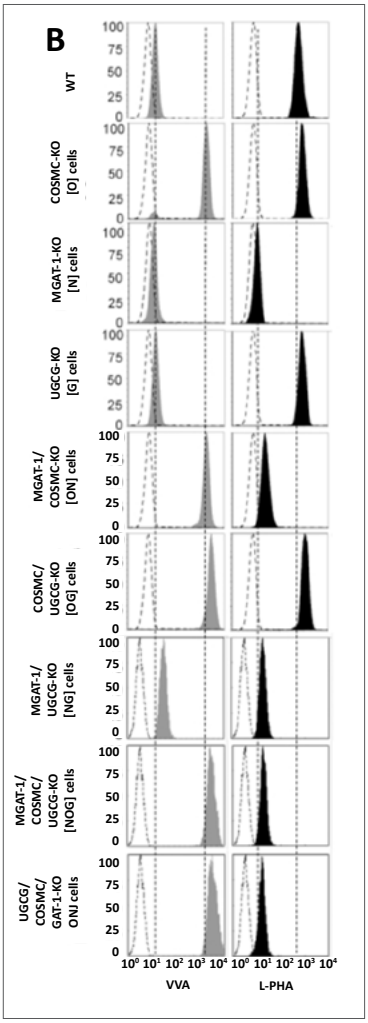
- CFB Solution can be used as a block and diluent if required.

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 min at 4C 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 anti-viral 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.

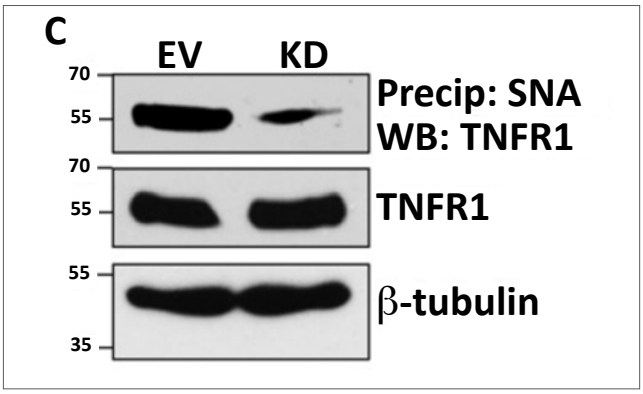
Incubate with agarose-bound lectin

For Batch

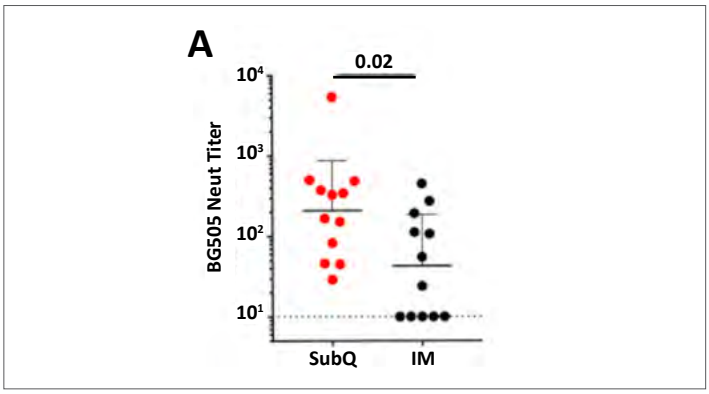
- Gently agitate sample with agarose lectin resin for 2-4 hours at room temperature.
  - » *Alternatively, incubate overnight at 4°C*
- Gently spin down beads (<1,000 *g*) and remove supernatant
- Wash 3x with wash buffer
- Incubate with 2x bead volume of appropriate eluting solution to your lectin column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

For Column

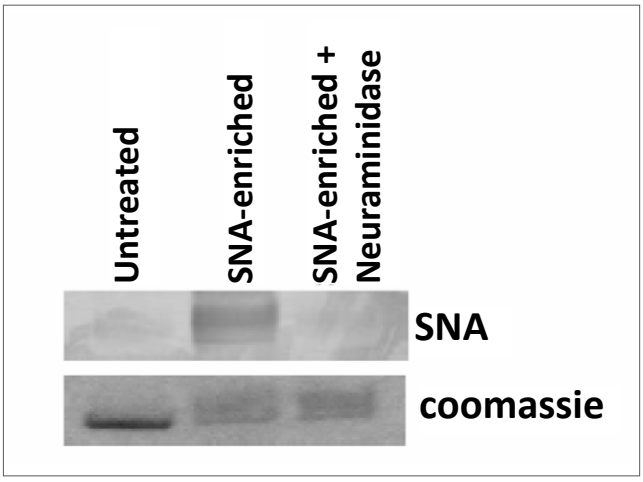
- Use gravity to slowly pull the glycoprotein sample through the column. Use of pressure will compress the beads and reduce binding. Depending on sample requirements, room temperature or cold room conditions can be applied.
- Wash resin with 2-3 column volumes of buffer to remove unbound material.
-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



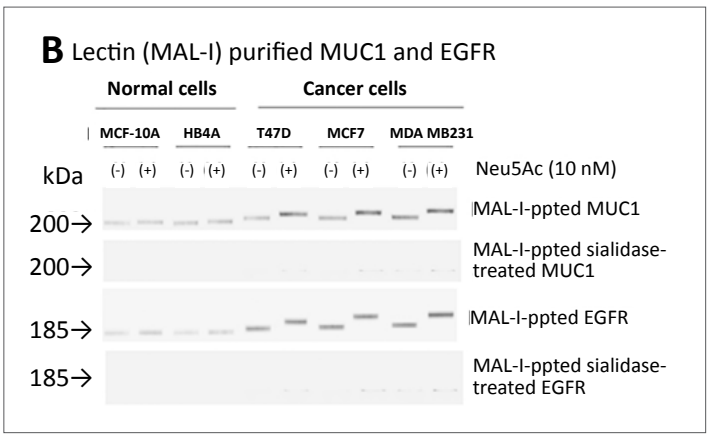
Determination of receptor glycosylation. Agarose SNA (Ref. 17).



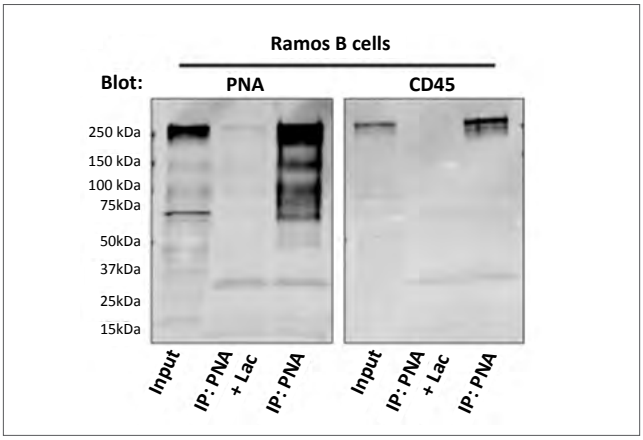
Immune response to an HIV glycopeptide purified by lectin chromatography. Agarose GNL (Fig. 1 - Ref. 10).



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



Examination of sialylation of MUC1 and EGFR on normal and malignant cells after sialic acid treatment under nutrient deprivation. FITC MAL 1 (Ref. 11).



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

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

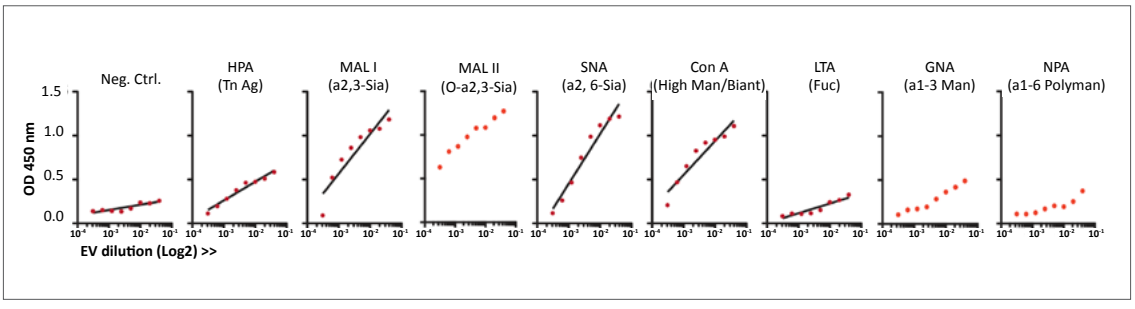
- Incubate plate with biotinylated lectin for 60 minutes at 4°C to room temperature (user optimized), in CFB Solution. Suggested initial working range is 0.5 – 10 mg/mL, user optimization required.
- Wash plate with PBST – **perform four times (x4).**

Visualization

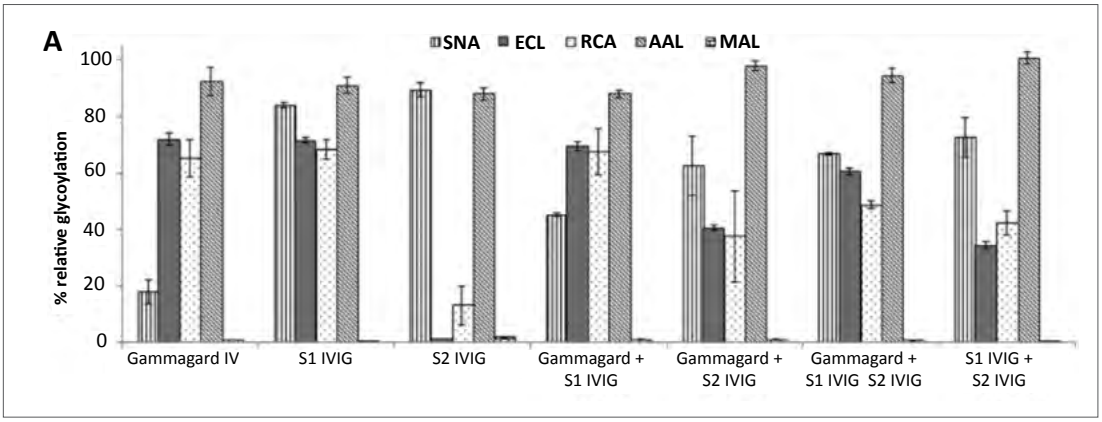
- Incubate plate with with enzyme-linked streptavidin or avidin as per manufacturer's instructions.
- Wash cells with PBST – **perform four times (x4).**
- Apply appropriate enzyme substrate solution.

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 LTA, Biotinylated GNA, Biotinylated NPA (Fig. 2 - Ref. 18).



Screening IVIG Fc fragment glycosylation via lectin ELISA. Biotinylated SNA; Biotinylated ECL; Biotinylated RCA; Biotinylated AAL; 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 39).

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

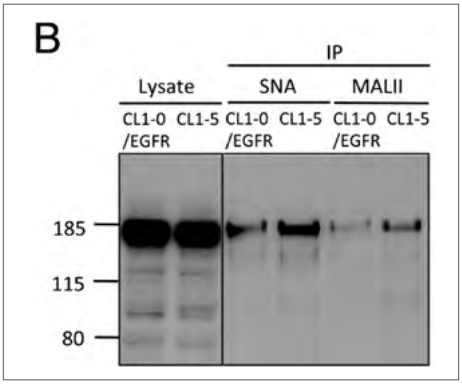
- Probe membrane with biotinylated lectin for 1 hour at room temperature, in CFB Solution. Suggested initial working range is 0.5 – 10 mg/mL, user optimization required.
- Wash membrane with TBST 5-10 minutes at room temperature – **perform four times (x4).**

Visualization

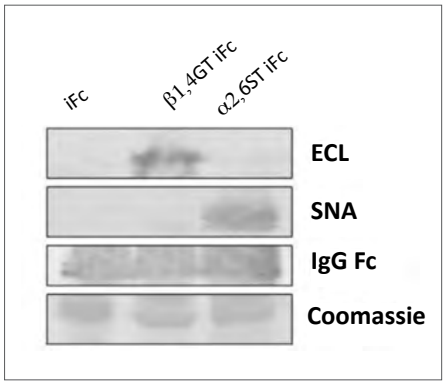
- Incubate membrane with streptavidin conjugated enzyme or VECTASTAIN ABC reagent for 1 hour at room temperature, in CFB Solution. Vector Laboratories offers a wide variety of HRP and AP reagents to support your needs.
- Wash membrane with TBST 5-10 minutes at room temperature – **perform four times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

Signal Acquisition

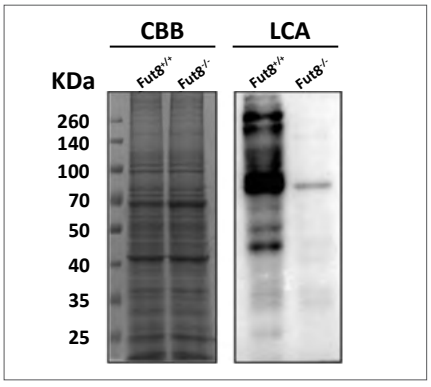
- Apply chemiluminescent or colormetric enzyme substrate following procedural time lines.
- Use imager or film to capture image of membrane.



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 – 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)

Procedure

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

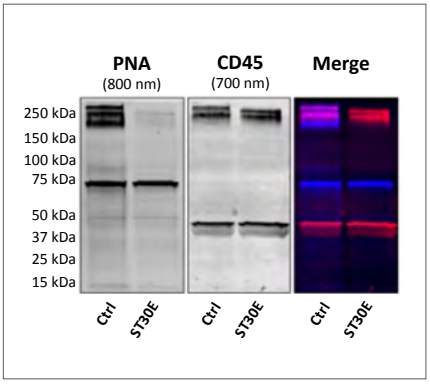
- Probe membrane with fluorophore-conjugated lectin for 1 hour at room temperature in CFB Solution. An alternative approach would be to use a biotinylated lectin. Suggested initial working range is 0.5 – 10 mg/mL, user optimization required.
  - » *Alternatively, probe overnight at 4°C.*
- Wash membrane 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

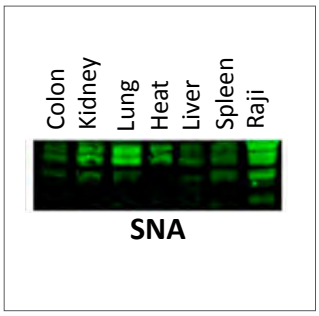
- If a biotinylated lectin was used incubate for 1 hour with fluorophore-conjugated avidin or streptavidin.
- Wash membrane with TBST 5-10 minutes, rocking, room temperature – **perform 2 times (x2).**

Signal Acquisition

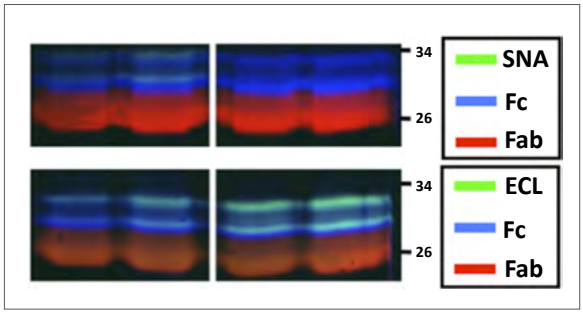
- Dry membrane.
- Image and analyze data.



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 the 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. Glycosyltransferase 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 a length of 2-4 hours at a temperature 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

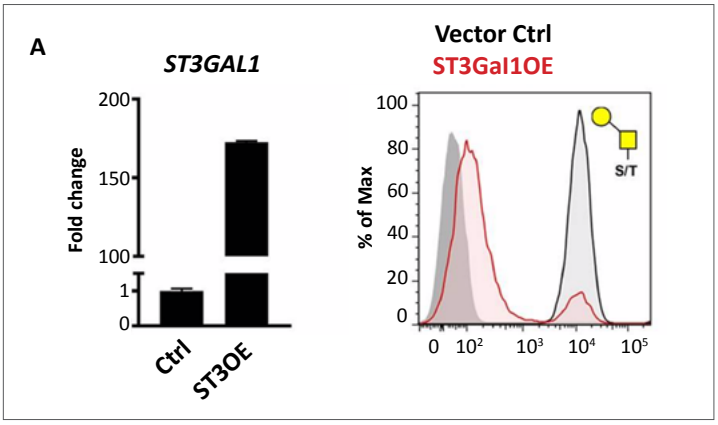
- Probe reactant with a fluorophore-labeled lectin for 1 hour at room temperature in CFB Solution. An alternative approach would be to use a biotinylated lectin.
  - » *Alternatively, probe overnight at 4°C.*
- Wash with a compatible wash buffer room temperature – **perform three times (x3).**

Visualization

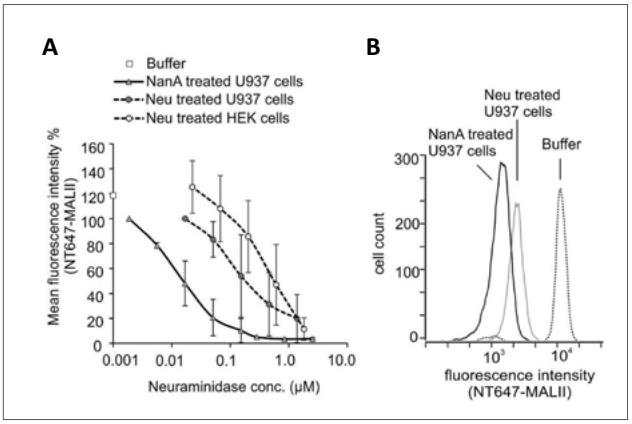
- If a biotinylated lectin was used, fluorophore-conjugated streptavidin would be applied at this point.
- Wash with a compatible wash buffer room temperature – **perform two times (x2).**

Signal Acquisition

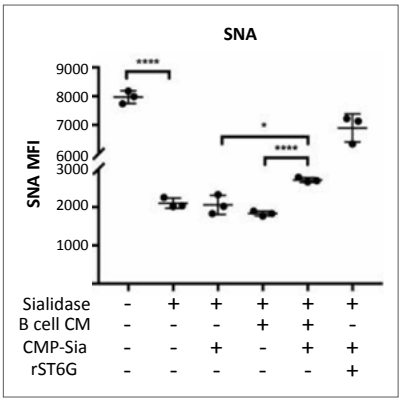
- Image and quantitate signal.



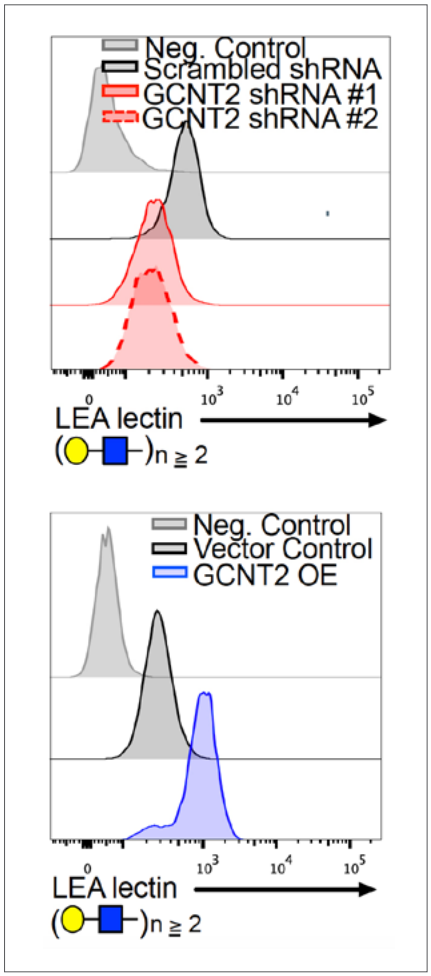
Loss of acceptor resulting from overexpression of an active glycosyltransferase. Biotinylated PNA (Fig. 2 - Ref. 20).



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).

Procedural Overview (Adapted from Ref. 13)

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

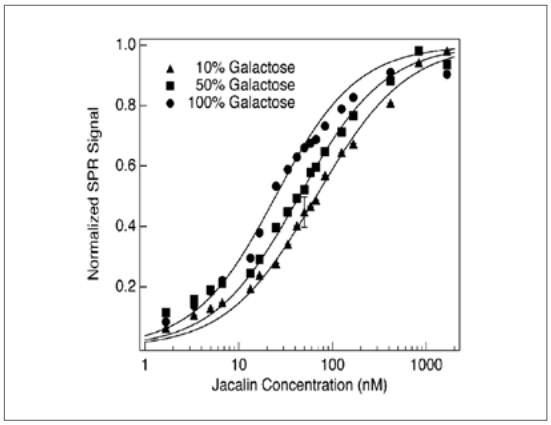
- Block the active flow chamber with 1.0 M ethanolamine/HCl, pH 8.5 for 10 minutes.
- Wash with running buffer.

Reaction and Detection

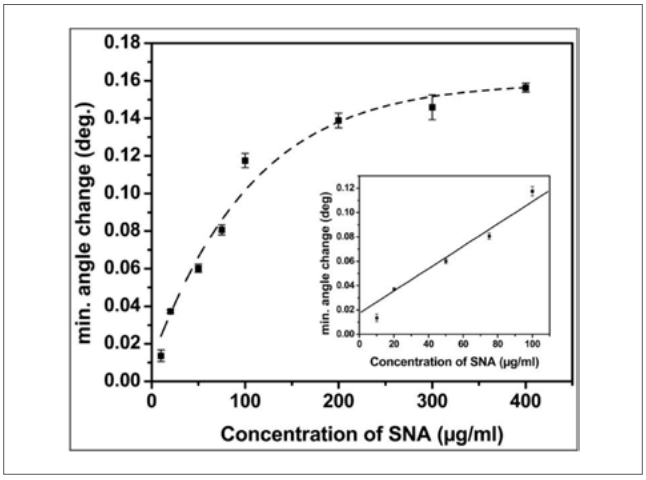
- Run unconjugated lectins across flow chamber at 10 µL per minute for 3 minutes at room temperature (21-24°C). We suggest using CFB Solution as a sample buffer.
- Regenerate chip surface with 10 mM glycine pH 2.0 and 10 mM NaOH for 1 minute between each lectin concentration to remove any binding from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

Select Published Applications

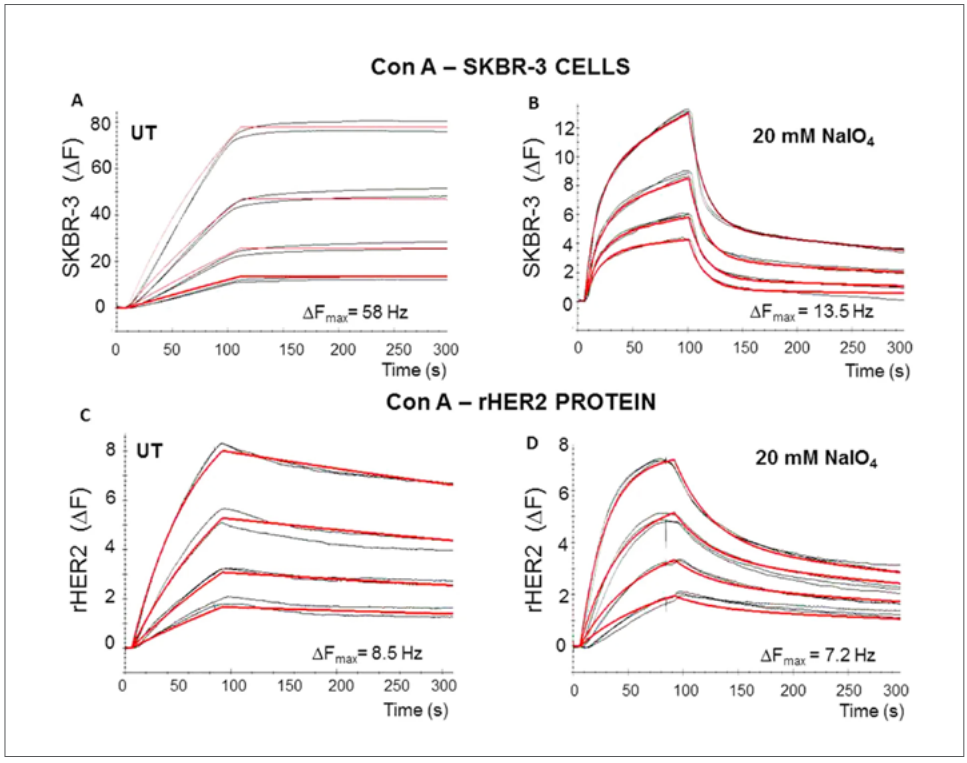
- Discerning the glycans on a target molecule (such as antibodies) (Refs. 12 and 13)
- Probing the binding specificity or behavior of a lectin (Refs. 25 and 26)
- Disease specific biomarker and bioanalytic testing (Ref. 50)



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 (lymphocyte) Proliferation

Assay:

To a population of cultured human T cells, incubate with a range (e.g. 5-50 µg/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.

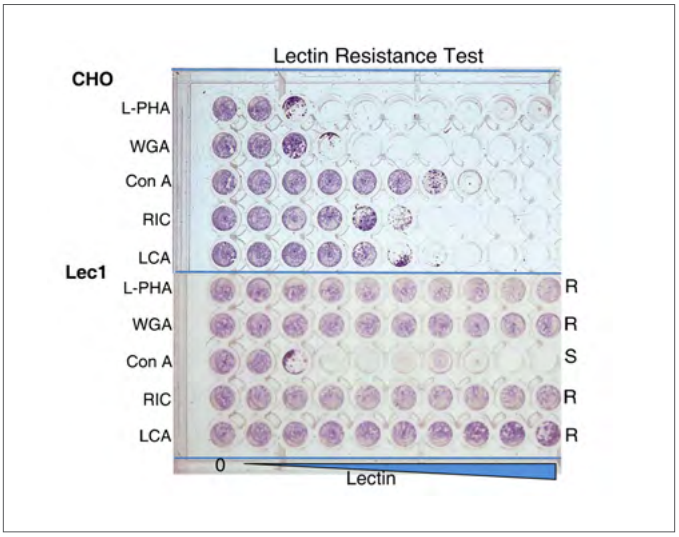
Cytotoxicity

Assay:

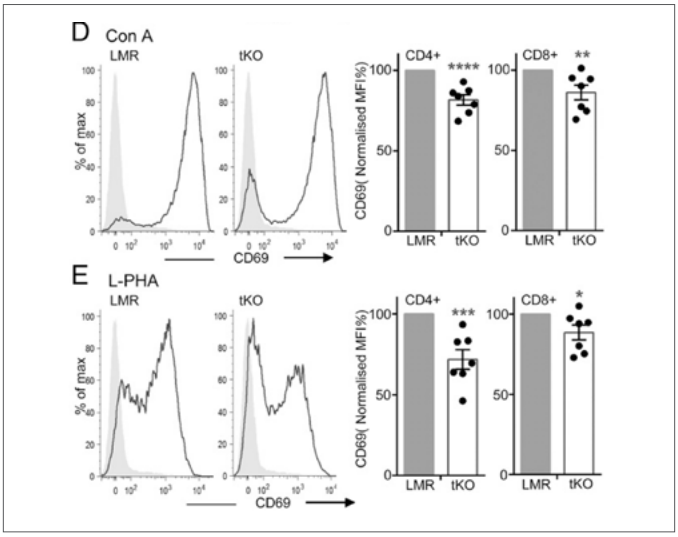
For a cell culture application, the methodology is fairly straightforward, whereby a specific lectin is added to the culture medium at varying concentrations and evaluated at multiple time points. Establishing a known model system of lectin toxicity against a given cell line will be helpful in streamlining 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:

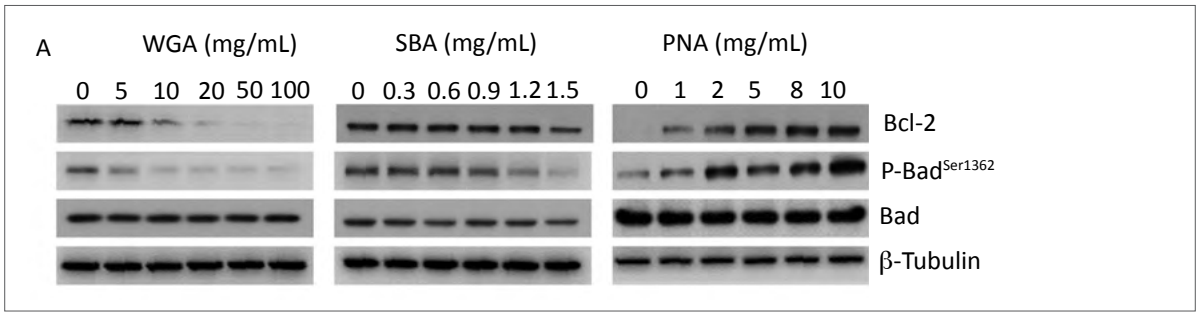
For lectin resistance type assays as outlined by Stanley (Ref. 46), toxicity was gauged by staining remaining live cell populations or by determining cellular enzyme activity. Depending on assay parameters, alternative procedures, such as the use of specific markers combined with platforms, could be 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 Copyright © 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. Copyright © (2015) The American Association of Immunologists, Inc.



Plant lectins 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.

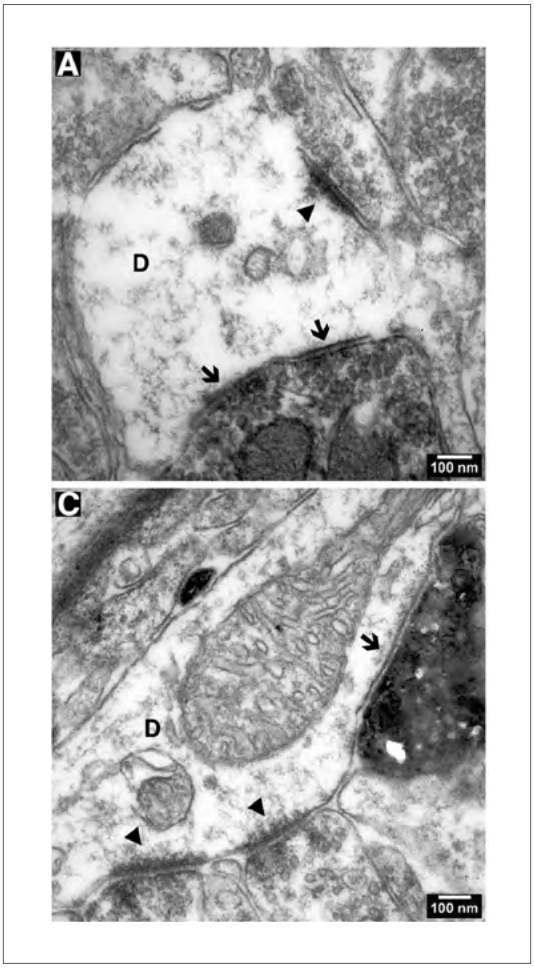
For a more comprehensive review of neural tracing techniques, see Vercelli et.al. (Ref. 88).

Select Published Applications

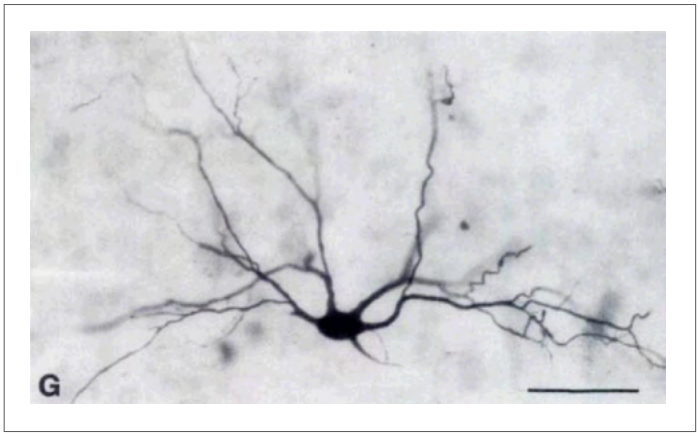
- Visualization of anterograde neuronal transport (Refs. 1 and 2)
- Determination of neuron-cell interaction (Ref. 16)
- Tracking transneuronal transport of viral particles (Ref. 55)
- Observing neuron-neuron interactions (Ref. 34 and 111)
- Demonstrate specific protein expression of neurons (Ref. 35)

Procedural Overview (Refer to Ref. 106)

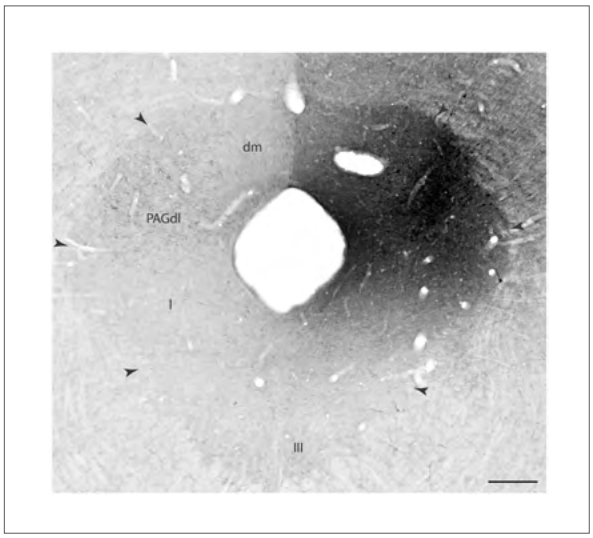
For PHA-L, please refer to "PHA-L Method for Tracing Efferent Neuronal Projections".



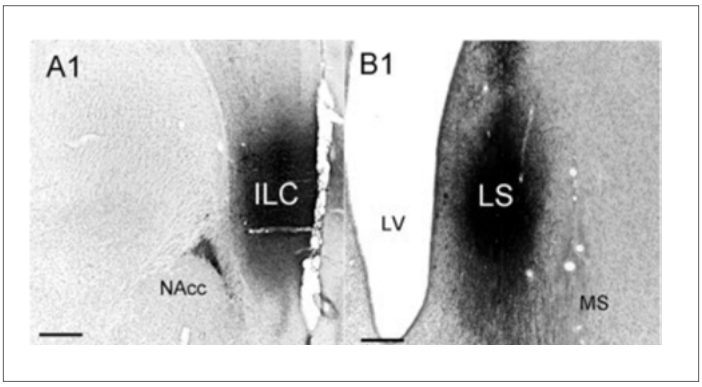
EM images of synapse formation in the CA1 region from PHA-L infused mice. Synaptic junctions indicated by arrows. Unconjugated PHA-L; Rabbit anti-PHA-(E+L); Goat anti-PHA-(E+L) (Fig. 7 - Ref. 104)



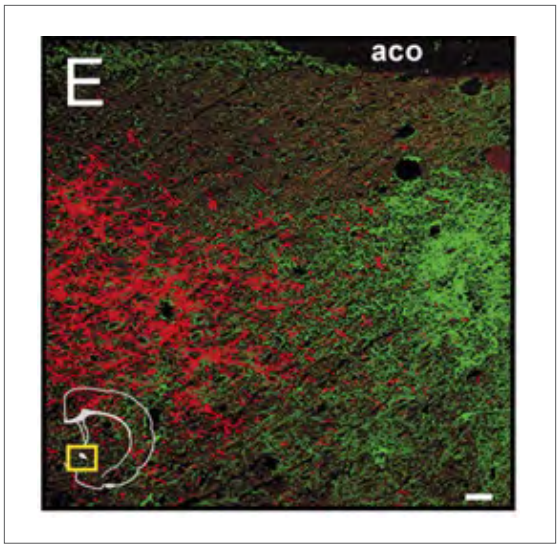
PHA-L labeling of ganglionic neuron of frog optic tectum. Unconjugated PHA-L (Fig 3G. Ref. 1)



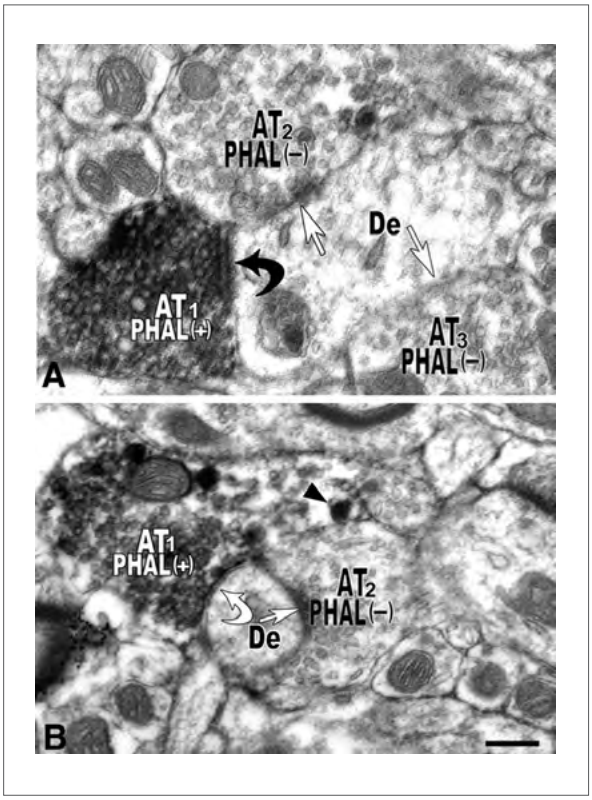
Neuronal labeling of an injection site at the dorsolateral column of the periaqueductal gray. Unconjugated PHA-L (Fig. 6 - Ref. 106).



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).



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).

Procedural Overview

Prepare lectin solution

- Dissolve/dilute lectin in sterile PBS at 0.5-2mg/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 uL).
- Apply gentle pressure to close injection site .

Sacrifice*

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

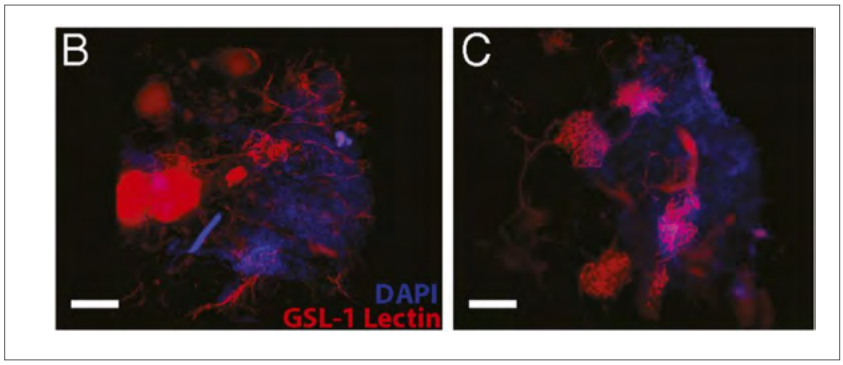
Tissue Preparation and Imaging

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

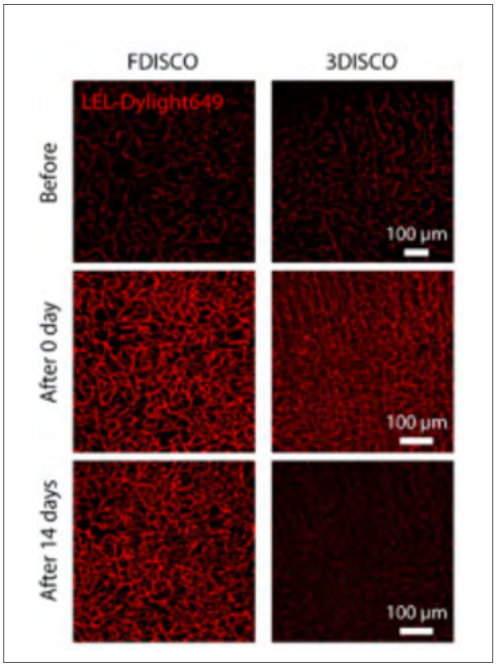
**All animal handling and techniques must be enacted in compliance with IACUC guidelines and with a protocol approved by an institutional animal facility.*

Select Published Applications

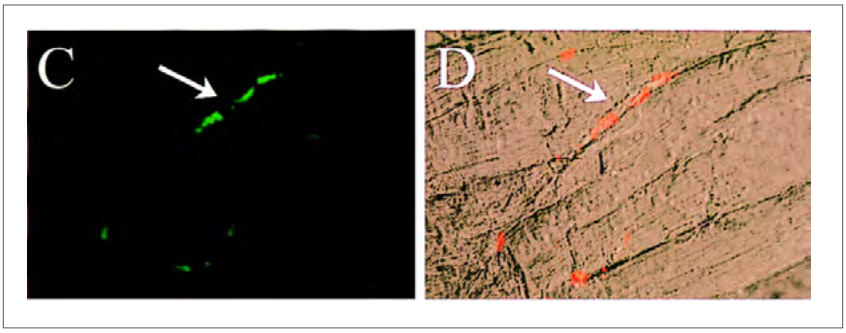
- Imaging host vascularization of engrafted tissues (Ref. 5)
- Analysis of vascular permeability and visualization of microvessels (Ref. 23)
- Quantifying tissue vascularization for tissue engineering (Ref. 33)
- Detection and quantification of tumor angiogenesis (Ref. 21)
- Determination of drug delivery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



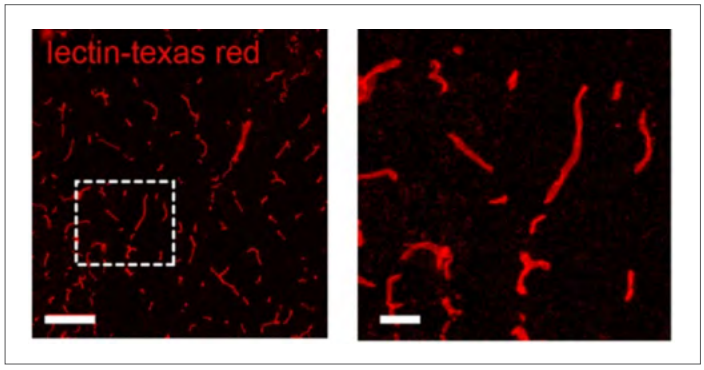
Monitoring vascularization of implanted islet cells embedded within endothelized modules. Unconjugated GSL I. (Fig. 3 - Ref. 107).



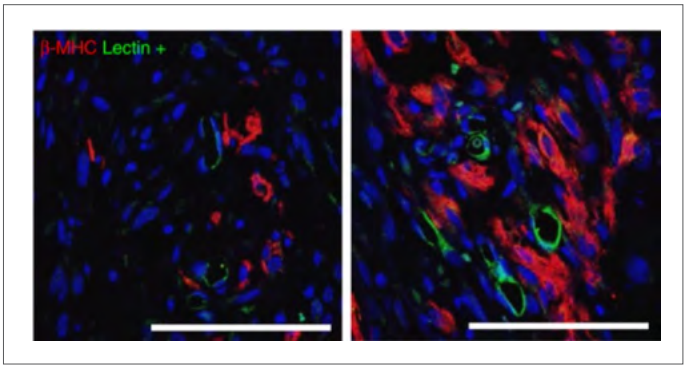
Comparison of optical clearing methods in mouse brain. DyLight 649 LEL (Fig. 2F - Ref. 27).



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



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

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 Laboratories. 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 you are studying:

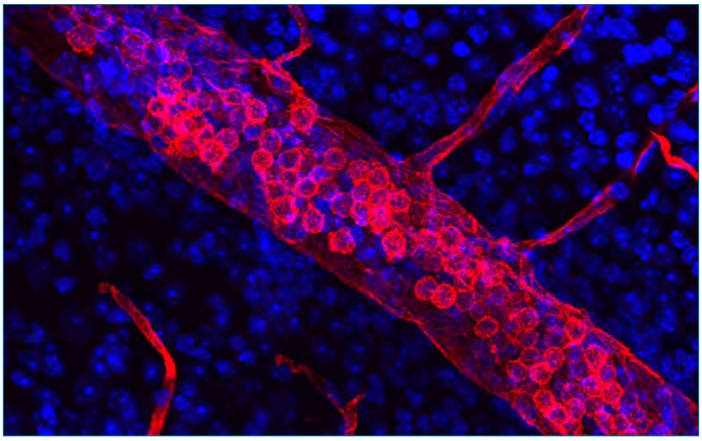
- Binding specificity of each lectin, for each of the hundreds of glycan structures in the array
- Data tabulated and conveniently summarized as a binding array, with peak height indicating degree of binding for each glycan
- Specific and detailed data on complex carbohydrate structure
- Lot-specific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

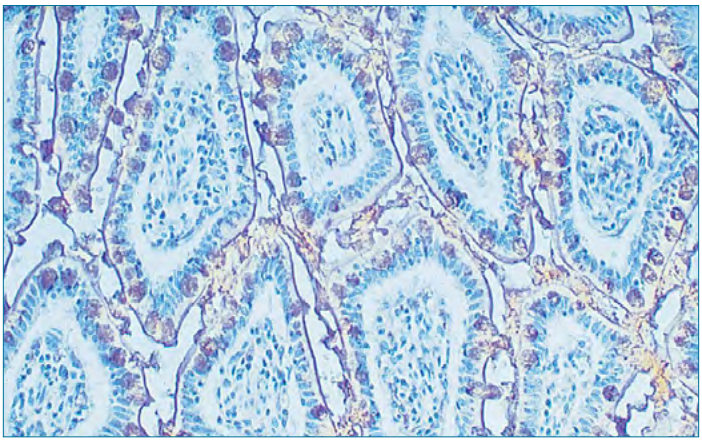
National Center for Functional Glycomics
NCFG

Summary of NCFG Services

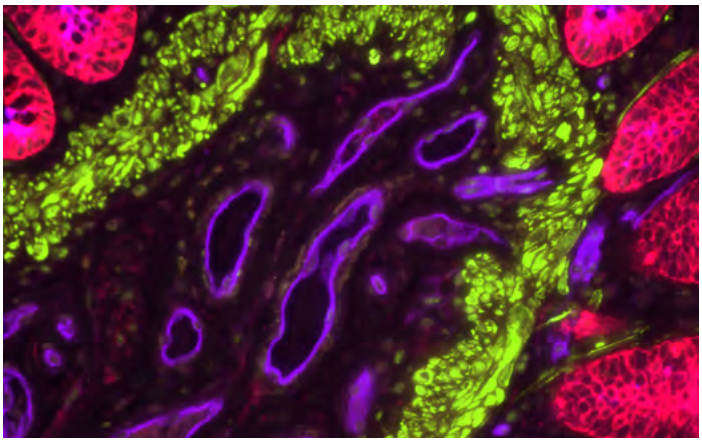
- Analysis of carbohydrate structures from glycoproteins via mass spectrometry
- Determining lectin glycan binding specificity of a sample against:
  - Defined glycan microarrays
  - Naturally derived shotgun glycan microarrays
- Custom array design and printing
- And more! Contact the NCFG to further 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.

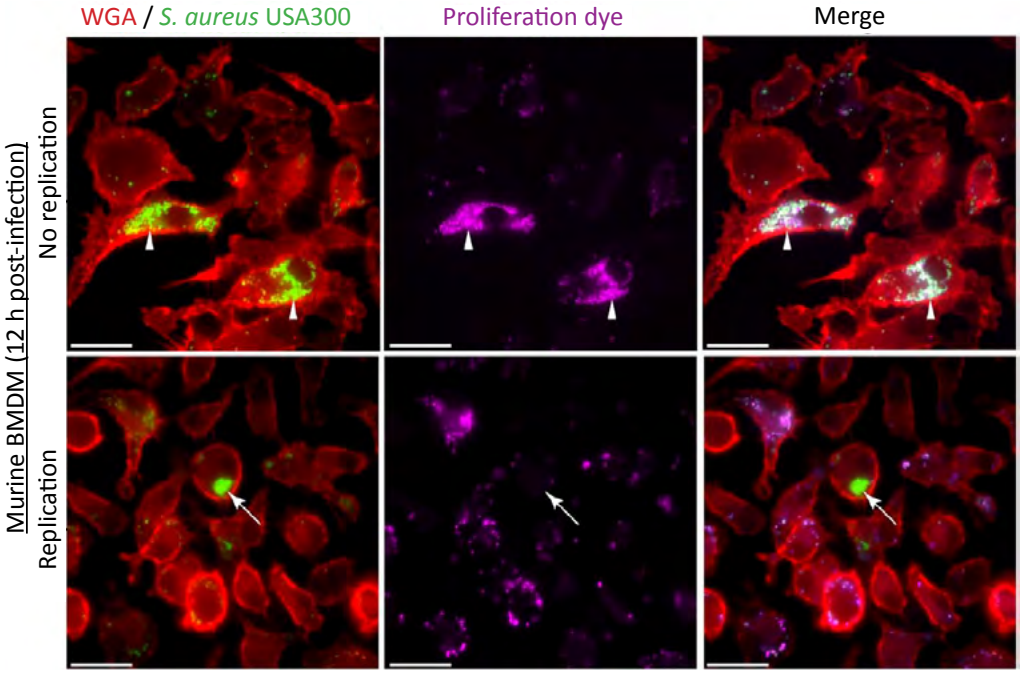


Human Colon: Rabbit Anti-Cytokeratin (AE1/AE3) and Mouse Anti-Desmin detected simultaneously with VectaFluor™ Duet Double Labeling Kit; Vasculature detected using DyLight 649 UEA I Lectin (purple). Mounted in VECTASHIELD PLUS Antifade Mounting Medium.

Other Lectin Applications

Bacterial Assays

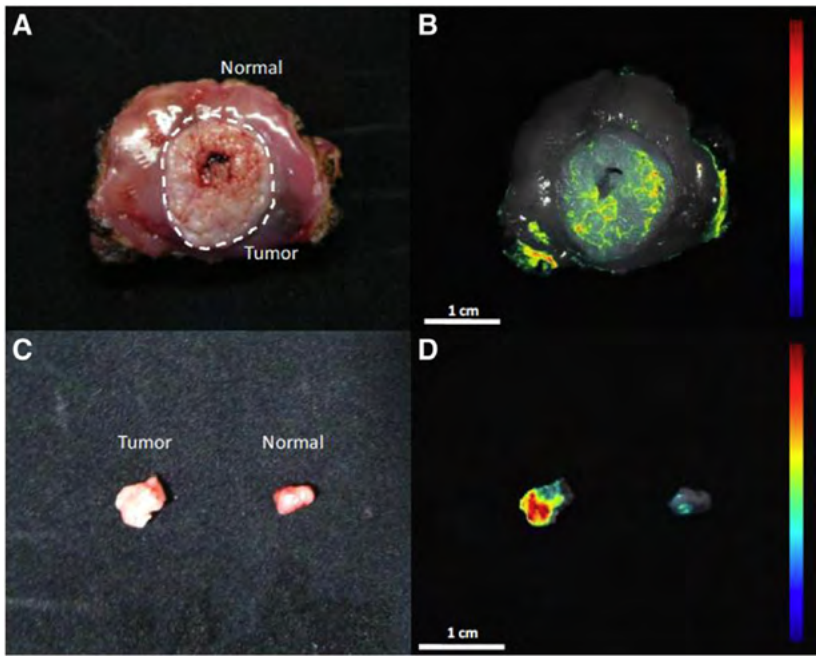
Fluorescent counterstain for intracellular bacterial growth assay



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

Cancer Markers

Multispectral epi-illumination to distinguish normal from cancerous



Directly labeled WGA staining differentiates cancerous versus normal tissue. (Fig. 2 - Ref. 40).

Lectins Used for Coronavirus Research

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].

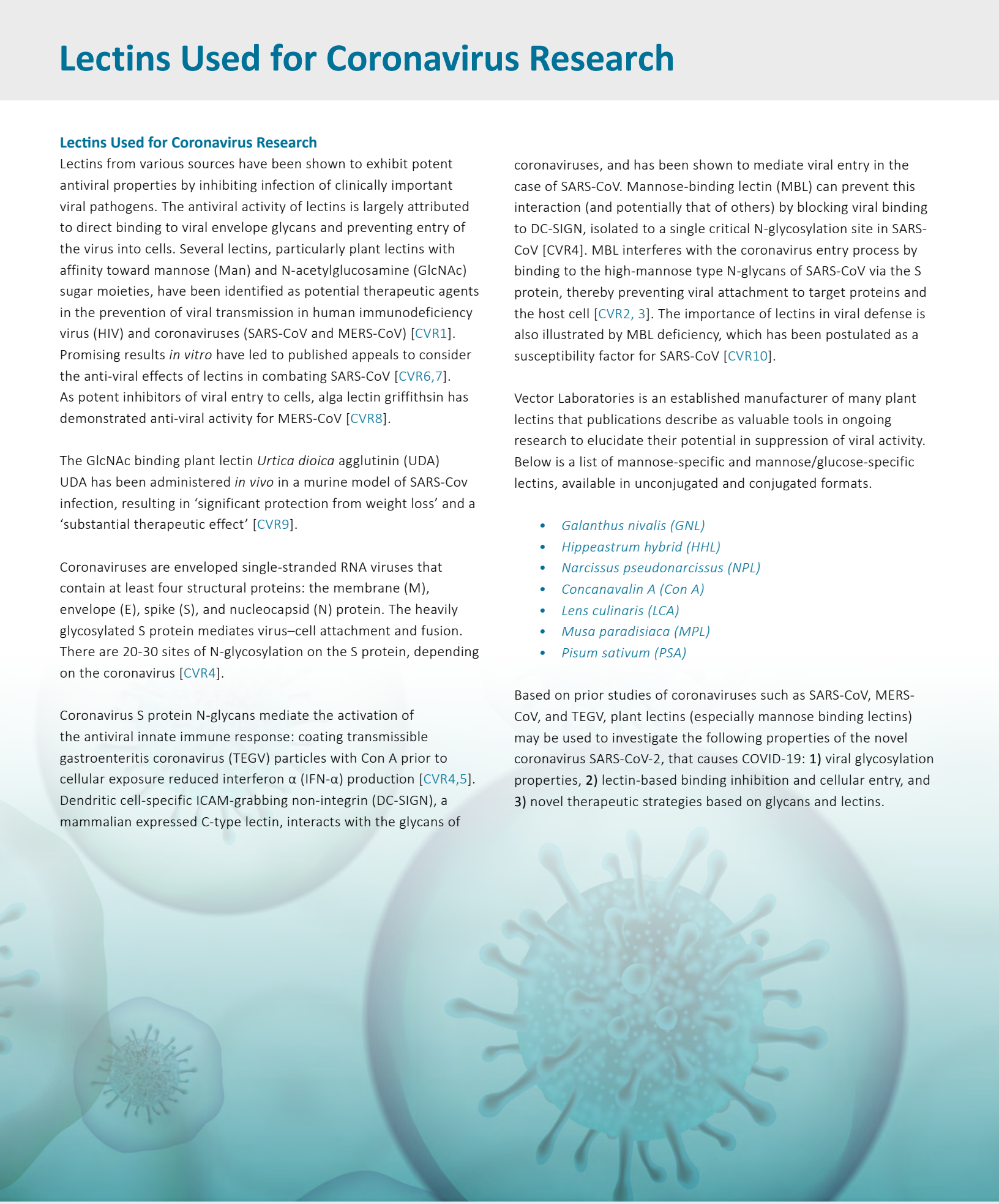
Coronaviruses are enveloped single-stranded RNA viruses that contain at least four structural proteins: the membrane (M), envelope (E), spike (S), and nucleocapsid (N) protein. The heavily glycosylated S protein mediates virus–cell attachment and fusion. There are 20-30 sites of N-glycosylation on the 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 Con A prior to cellular exposure reduced interferon α (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)*
- *Hippeastrum hybrid (HHL)*
- *Narcissus pseudonarcissus (NPL)*
- *Concanavalin A (Con A)*
- *Lens culinaris (LCA)*
- *Musa paradisiaca (MPL)*
- *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. Antiviral Lectins: Selective Inhibitors of Viral Entry. Antiviral Res. 2017 Jun; 142: 37–54. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5414728/)

CVR2. Keyaerts, E. et al. 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. (https://www.sciencedirect.com/science/article/pii/S0166354207002380?via%3Dihub)

CVR3. Ritchie, G., et al. Identification of N-linked carbohydrates from severe acute respiratory syndrome (SARS) spike glycoprotein. Virology. 2010 Apr 10;399(2):257-69. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3412594/)

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

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

CVR6. Mazalovska M, Kouokam JC. Lectins as Promising Therapeutics for the Prevention and Treatment of HIV and Other Potential Coinfections. Biomed. Res. Int. 2018 May 8;2018:3750646. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5964492/)

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

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

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

CVR10. Ip WK, Chan KH, Tso GH et.al. Mannose-binding lecting in severe acute respiratory syndrome coronavirus infection. J Infect Dis. 2005 May 15;191(10):1697-704. (https://www.ncbi.nlm.nih.gov/pubmed/15838797)

Table of Lectin Properties

Table of Lectin Properties

Lectin	Common Name	Source	Glycoprotein	Metal Ions Present	Mitogenic Activity	Blood Group Specificity	Preferred Sugar Specificity	Inhibitor or Eluting Sugar
Agaricus bisporus	ABL	*Agaricus bisporus* white button mushrooms	–	No	No	Non-specific	Gal(β-1,3) GalNAc	Fetuin
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	$(GlcNAc)_{2-4}$	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
Hippeastrum hybrid	HHL, AL	*Hippeastrum hybrid* (Amaryllis) bulbs	No	No	No	Rabbit	αMan	MeαMan
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	$(GlcNAc)_{2-4}$	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
Musa paradisiaca	BanLec	*Musa paradisiaca* (banana fruit)	–	–	Yes	Rabbit	αMan, αGlc	MeαMan
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
Ricin A Chain	Ricin A Chain	RCA_{60}	Yes	No	No	–	–	–
Ricin B Chain	Ricin B Chain	RCA_{60}	Yes	No	Yes	–	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 *a-Methylmannoside*
Neu5Ac *N-Acetylneuraminic acid (sialic acid)*
SA *Sialic Acid*

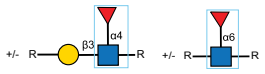
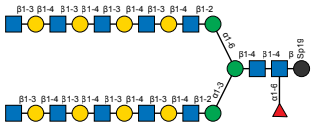
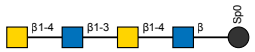
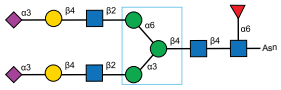
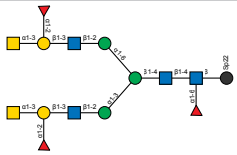
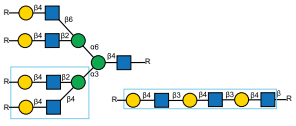
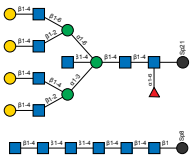
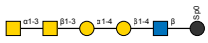
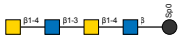
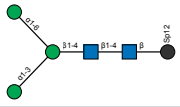
Lectin Properties with Structures

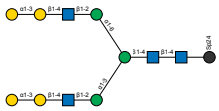
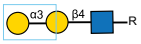
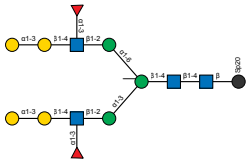
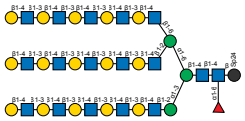
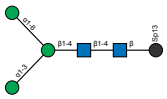
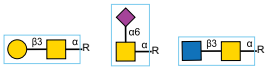
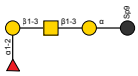
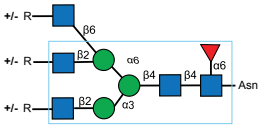
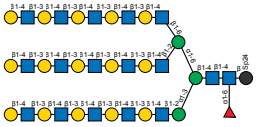
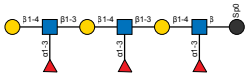
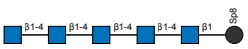
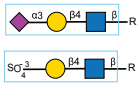
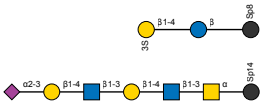
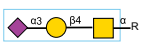
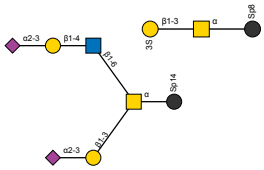
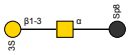
Adapted from Essentials of Glycobiology 3e, with permission from The Consortium of Glycobiology Editors, La Jolla, California; published by Cold Spring Harbor Laboratory Press

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

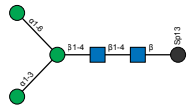
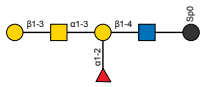
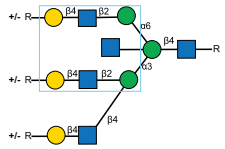
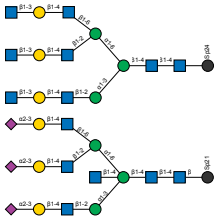
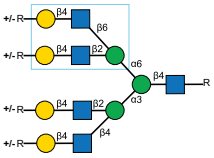
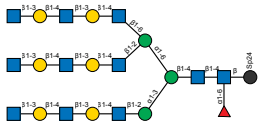
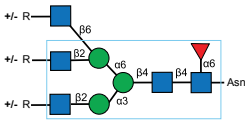
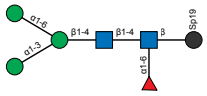
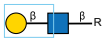
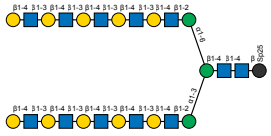
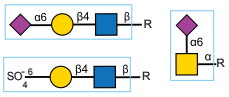
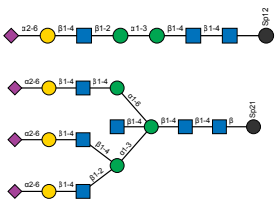
Each lectin is a unique protein that has evolved to interact with distinct carbohydrate epitopes. Deriving from many different species, if grouped by the terminal sugar that the lectin generally binds, each will differ in the strength of binding due to affinity and avidity for the epitope. This may be 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 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).

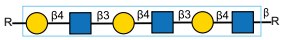
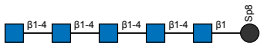
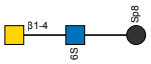
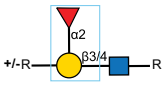
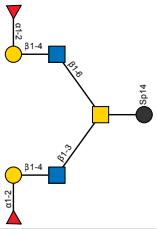
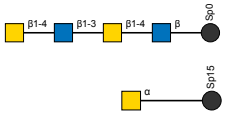
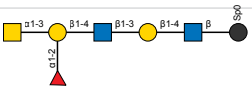
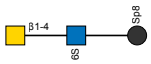
Preferred Glycan Binding Partners and Inhibiting Sugars

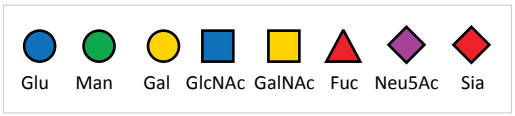
Lectin	Name, Abridged	Inhibitory Sugar[1]	Rough Sugar Specificity[1]	General Binding Motif[2]	Data Source	Top Structure on Array[3]
Agaricus bisporus	ABL	Gal	O-linked Gal β-1, 3 GalNAc	—	—	—
Aleuria aurantia	AAL	Fuc	Fuc		Data Link	
Bauhinia purpurea	BPL, BPA	Gal	Primanily Gal β-1,3 or 1,4 but will also 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 (Bandelraea) 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	
Hippeastrum hybrid	HHL, AL	Man	α-1,3 Mannose and α 1,6 mannose	—	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-acetylgluco-samine, (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	
Musa paradisiaca	BanLec	Man	αMan, αGlc	—	—	—

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 hypogaea)*	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	
Ricin A Chain	Ricin A Chain	—	—	—	—	—
Ricin B Chain	Ricin B Chain	Lac	β-Gal/GalNAc	—	—	—
Sambucus nigra	SNA, EBL	Lac	α-1,6 sialic acid-LacNAc structure		Data Link	

Lectin	Name, Abridged	Inhibitory Sugar[1]	Rough Sugar Specificity[1]	General Binding Motif[2]	Data Source	Top Structure on Array[3]
Solanum tuberosum	STL, PL	GlcNAc	GlcNAc oligomers, LacNAc		Data Link	
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. (Ref. 95)
2. Essentials of Glycobiology (Ref. 67)
3. Structures drawn with GLAD (Ref. 96)
4. Symbol Nomenclature for Glycans (Ref. 97)

Lectin Products/SKU Table

Agaricus Bisporus Lectin (ABL)		
Unconjugated	L-1420	2 mg
Aleuria Aurantia Lectin (AAL)		
Unconjugated	L-1390	2 mg
Agarose (2 mg lectin/mL gel)	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 (6 mg lectin/mL gel)	AL-1003	10 mL, 100 mL
Biotin	B-1005	5 mg
CY3	CL-1003	1 mg
Fluorescein	FL-1001	25 mg
Rhodamine	RL-1002	25 mg
Datura Stramonium Lectin (DSL)		
Unconjugated	L-1180	5 mg
Agarose (3 mg lectin/mL gel)	AL-1183	2 mL
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-1031	2 mg, 5 mg
Rhodamine	RL-1032	2 mg
Erythrina Cristagalli Lectin (ECL, ECA)		
Unconjugated	L-1140	10 mg
Agarose (3 mg lectin/mL gel)	AL-1143	2 mL
Biotin	B-1145	5 mg
Fluorescein	FL-1141	5 mg

Galanthus Nivalis Lectin (GNL)		
Unconjugated	L-1240	5 mg
Agarose (3 mg lectin/mL gel)	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
Agarose (3 mg lectin/mL gel)	AL-1213	2 mL
Biotin	B-1215	2 mg
Fluorescein	FL-1211	2 mg
Hippeastrum Hybrid (Amaryllis) Lectin (HHL, AL)		
Unconjugated	L-1380	5 mg
Biotin	B-1385	2 mg
Jacalin		
Unconjugated	L-1150	25 mg
Agarose (4 mg lectin/mL gel)	AL-1153	10 mL
Biotin	B-1155	5 mg
Fluorescein	FL-1151	5 mg

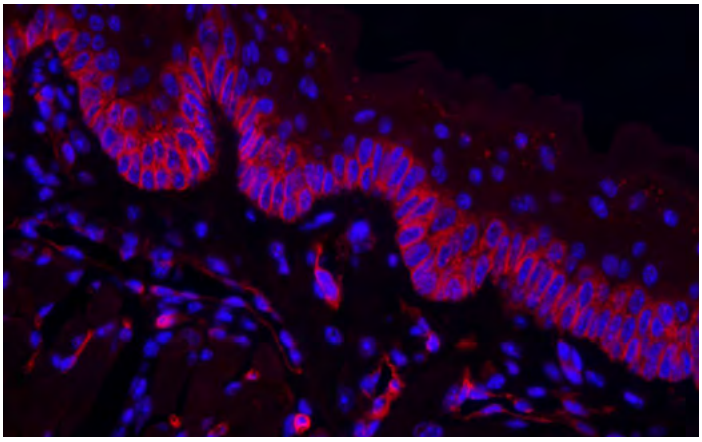
Lens Culinaris Agglutinin (LCA, LcH)		
Unconjugated	L-1040	10 mg, 25 mg
Biotin	B-1045	5 mg
DyLight 649	DL-1048	1 mg
Fluorescein	FL-1041	5 mg
Rhodamine	RL-1042	5 mg
Lotus Tetragonolobus Lectin (LTL)		
Unconjugated	L-1320	5 mg
Biotin	B-1325	2 mg
Fluorescein	FL-1321	2 mg
Lycopersicon Esculentum (Tomato) 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
Biotin	B-1345	2 mg
Musa Paradisiaca Lectin (BanLec)		
Unconjugated	L-1410	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 (5 mg lectin/mL gel)	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 Agglutinin (PHA)		
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 (4 mg lectin/mL gel)	AL-1083	2 mL, 10 mL
Biotin	B-1085	5 mg
Fluorescein	FL-1081	5 mg
Rhodamine	RL-1082	5 mg
Ricin A Chain		
Unconjugated	L-1190	1 mg
Ricin B Chain		
Unconjugated	L-1290	1 mg

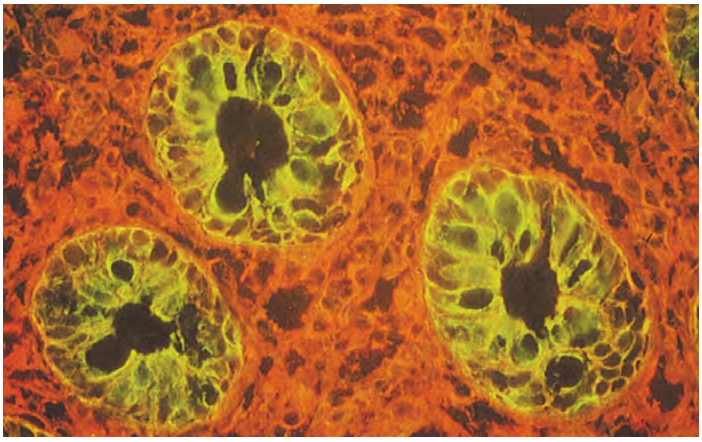
Lectin Products/SKU Table (continued)

Sambucus Nigra Lectin (SNA, EBL)		
Unconjugated	L-1300	5 mg
Agarose (3 mg lectin/mL gel)	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 (4 mg lectin/mL gel)	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 (2 mg lectin/mL gel)	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 (3 mg lectin/mL gel)	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 (7 mg lectin/mL gel)	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		
Unconjugated	L-1020S	10 mg
Agarose (3 mg lectin/mL gel)	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 (3 mg lectin/mL gel)	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).



Frozen section of colon viewed under fluorescein filter: Lycopersicon esculentum lectin (orange staining with Vector Red) and glandular epithelium (green fluorescein label).

Lectin Kits and Select Ancillary Products

Lectin Screening Kits I		
Biotinylated Lectin Kit I	BK-1000	1 kit
Fluorescein Lectin Kit I	FLK-2100	1 kit
Rhodamine Lectin Kit I	RLK-2200	1 kit
Lectin Screening Kits II		
Biotinylated Lectin Kit II	BK-2100	1 kit
Lectin Screening Kits III		
Biotinylated Lectin Kit III	BK-3000	1 kit

- **Kit I (BK-1000, FLK-2100, RLK-2200)** 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, FLK-4100)** consists of 0.5 mg of the following lectin conjugates: DSL, ECL, GSL II, Jacalin, LEL, STL, VVL.

Inhibiting Sugars

Product	Catalog Number	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	Catalog Number	Unit Size
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.*

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
TrueVIEW Autofluorescence Quenching Kit (No Counterstain)	SP-8400	1 Kit
TrueVIEW Autofluorescence Quenching Kit (with DAPI Counterstain)	SP-8500	1 Kit
VECTASHIELD Antifade Mounting Medium (non-setting formulation)	H-1000	10 mL
VECTASHIELD Antifade Mounting Medium with DAPI (non-setting 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-setting formulation)	H-1900	2 mL, 10 mL
VECTASHIELD PLUS Antifade Mounting Medium with DAPI (non-setting formulation)	H-2000	2 mL, 10 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
Fucose- or Arabinose-binding Lectins	ES-3100	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-3100	ES-5100	ES-7100
AAL (AL-1393)			+		
Con A (AL-1003)	+				
DSL (AL-1183)				+	
ECA (AL-1143)		+			
GNL (AL-1243)	+				
GSL II (AL-1213)				+	
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

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Contact Details

US Office

USA Headquarters:
Vector Laboratories, Inc.
30 Ingold Road
Burlingame, CA 94010
Tel: +1 (650) 697-3600
Ordering and Technical Service (USA): +1 (800) 227-6666
Customer Service: customerservice@vectorlabs.com
Technical Support: technical@vectorlabs.com
International Inquiries: Please contact your local distributor.

UK Office

United Kingdom:
Vector Laboratories Ltd.,
3 Accent Park, Bakewell Road, Orton Southgate
Peterborough, PE2 6XS
United Kingdom
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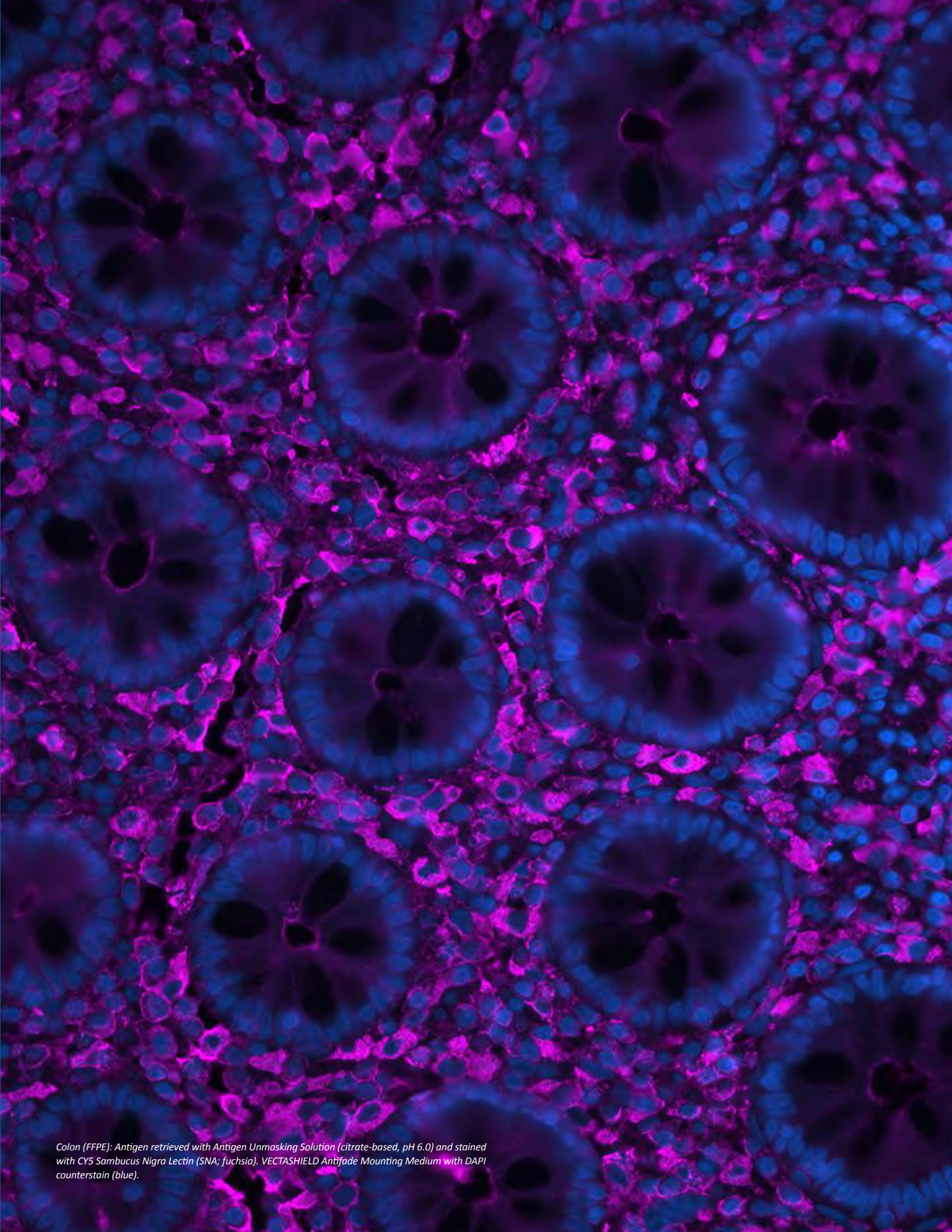

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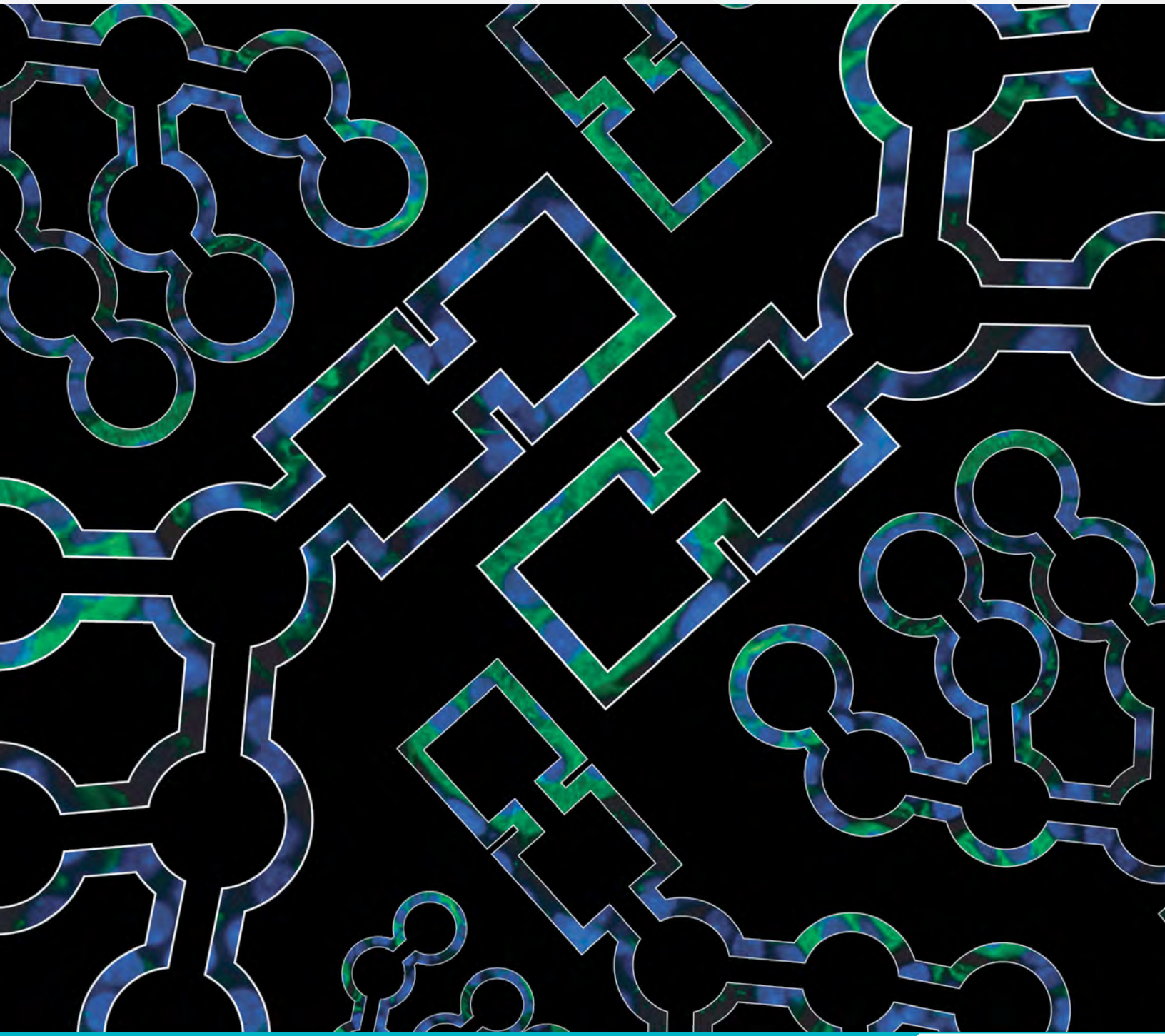

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