Last update: February 2016

Main applications of lectin-based microarray (GlycoStation™) for glycan profiling

- **Quick and simple glycan monitoring of therapeutic proteins such as biologicals, EPO, and other for high throughput glycan profiling.**
- **Anti-doping analysis e.g. EPO by taking advantage of glycan differences.**
- **Characterization of stem cell (ES, iPS, MSC etc.) in regenerative medicine.**
- **New glycan biomarker development and diagnosis.**
- **Glycan profiling of viruses, intestinal bacteria and analysis of infectability and immunity.**

Features & Benefits

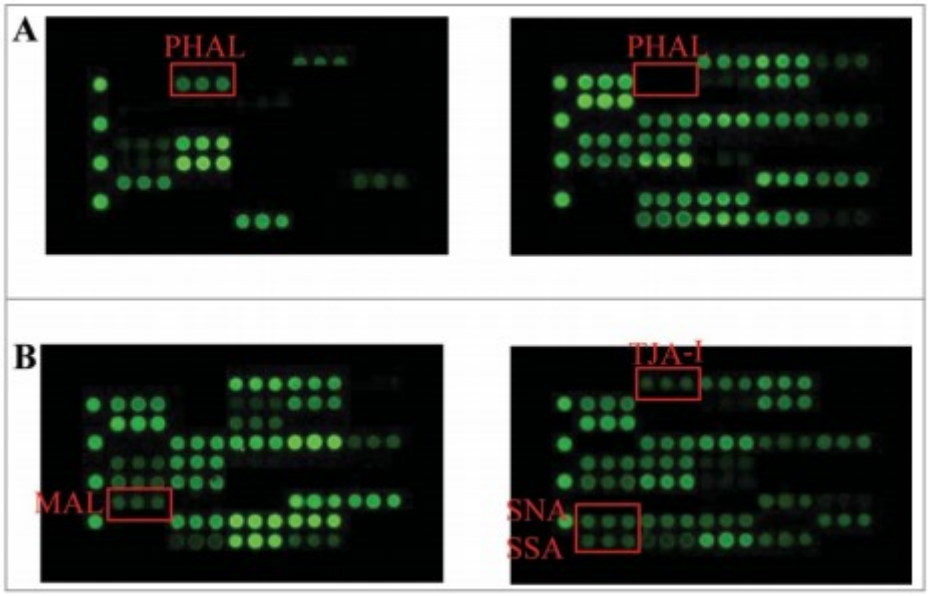
- Complementary to mass spectrometry and HPLC
- High sensitivity using low sample volumes
  (LOD: 100pg/ml glycoprotein, 100 pM glycan, 103 cells)
- Differential analysis of purified and crude samples
- Identification of N- and O-glycans
- Differentiation of isomers
- High throughput Screening - simple and quick to use
- Analysis of various samples types including crude samples - cell lysates, cell extracts

Introduction: GlycoStation™ based on Lectin Microarrays, is a versatile system for a simple and highly sensitive analysis of glycoproteins in various sample types (serum, plasma, cell culture, tissue-crude extracts..). Compared to MS-based methods, which usually involve multiple sample processing steps, a lectin microarray directly works on intact glycoproteins with only minimal alteration to a testing sample. Though LC-MS/MS is a powerful technology in identifying proteins and N-glycan structures, its low sensitivity has a certain disadvantage, since there is a reduction in sensitivity by a factor of 100, compared to the Lectin Microarray (GlycoStation™) Technology. Although GlycoStation™ cannot identify glycan structures perfectly, it is a powerful system to identify differences of glycan structures very sensitive from a small amount of sample. The lectin microarray method, is in comparison to other technologies, a very easy method to specifically identify glycan isomers, such as α2,3-Sia, α2,6-Sia. One important feature of the Lectin

Microarray (GlycoStation™) Technology to be able to gain data about O-glycans as well as N-glycans at the same time.

1. Glycan Analysis of Therapeutic Glycoproteins presented by FDA

Therapeutic monoclonal antibodies (mAbs) are glycoproteins produced by living cell systems. The glycan moieties attached to the proteins can directly affect protein stability, bioactivity, and immunogenicity. Therefore, glycan variants of a glycoprotein product must be adequately analyzed and controlled to ensure product quality. However, the inherent complexity of protein glycosylation poses a daunting analytical challenge. This review provides an update of recent advances in glycan analysis, including the potential utility of lectin-based microarray for high throughput glycan profiling. Emphasis is placed on comparison of the major types of analytics for use in determining unique glycan features such as glycosylation site, glycan structure, and content. Their data show promise for lectin microarrays in profiling glycan variants that are commonly present in therapeutic proteins. Compared to MS-based methods, which usually involve multiple sample processing steps, a lectin microarray directly works on intact glycoproteins with only minimal alteration to a testing sample (e.g., fluorescent labeling). The lectin microarray, when coupled with a sophisticated detection system, appears to provide a high throughput platform for rapid screening of glycan profiles of therapeutic proteins. Differences in glycan profiles can be easily shown as below.



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The use of lectin microarray for assessing glycosylation of therapeutic proteins.

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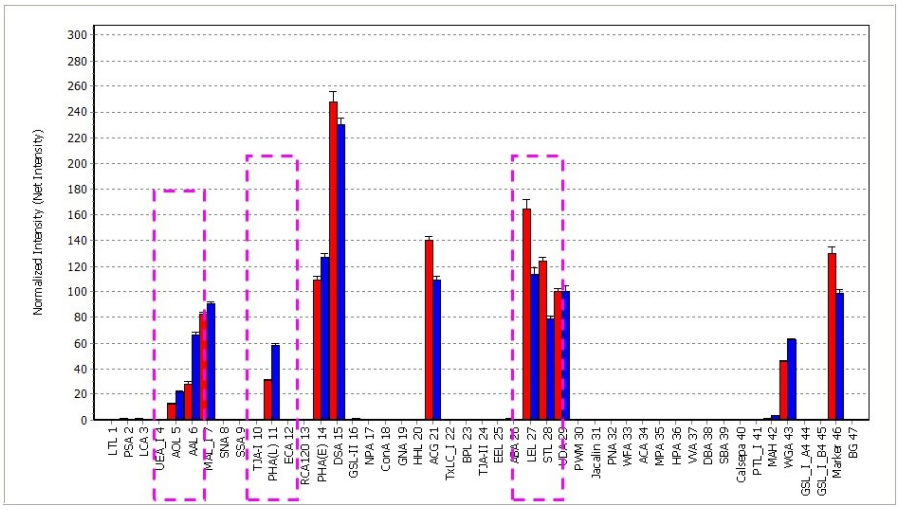
2. Glycan Profiling Analysis of EPOs

EPO (Erythropoietin) is a drug that improves anemia. It is well know that drug efficacy of biotechnology-based drugs such as EPO and IgG is greatly affected by the glycosylation. According to a paper published in Folio Pharmacol., Jpn, 131, pp192-199, the second generation EPO, in which glycosylation is modulated with the introduction of two additional N-glycans, has proven to have a higher hematopoietic effect in comparison to the first generation Epo drug. The figure below shows a comparison of glycan profiles between the fisrt generation and the second generation EPOs (red color shows the first generation and blue color shows the second generation). The difference shown by PHA-L suggests that tetra-antennary N-glycans are more expressed in the second generation, the strong signal of MAL_I indicates that those N-glycans are heavily terminated by &alpha2,3 Sia, the differences shown by AOL and AAL suggest that those N-glycans are more fucosylated in the second generation, and also differences are seen in polylactosamine binder LEL and lactosamin binder STL. Thus, we believe that lectin microarray is a very powerful tool in comparing differences of glycosylation among biotechnology-based drugs and useful in accelerating drug R&D. The technology also enables glycan profiling of secreted glycoproteins in cell culture supernatants.



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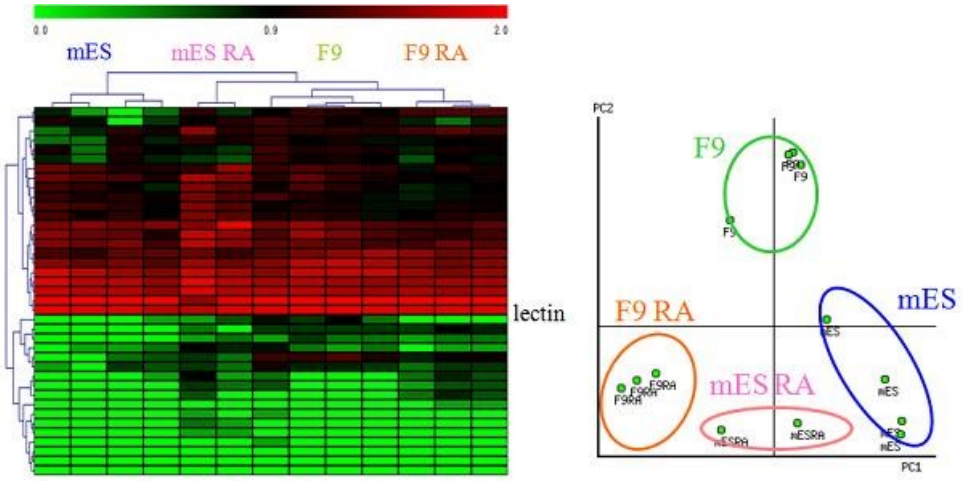
3. Characterization of Stem Cells（hES, iPS, MSC etc.）

It was shown that mES (mouse ES), F9 (mouse embryonic carcinoma cell) and differentiated cells by retinoic acid (RA) were clearly discriminated each other by applying hierarchical clustering and principal component analyses to those glycan profiling patterns using LecChip. This result was presented at ISSCR 2009 by Dr. Toyoda and Dr. Umezawa, NCCHD, et al. This method also applies to hES, iPS and MSC, and enables discrimination with a higher degree of accuracy than ever. This method was tested developmentally by the groups of AIST (Research Center for Stem Cell Engineering), CiRA (Kyoto University) and The Scripps Research Institute, et al, and also applied to cancer stem cells at Osaka University.



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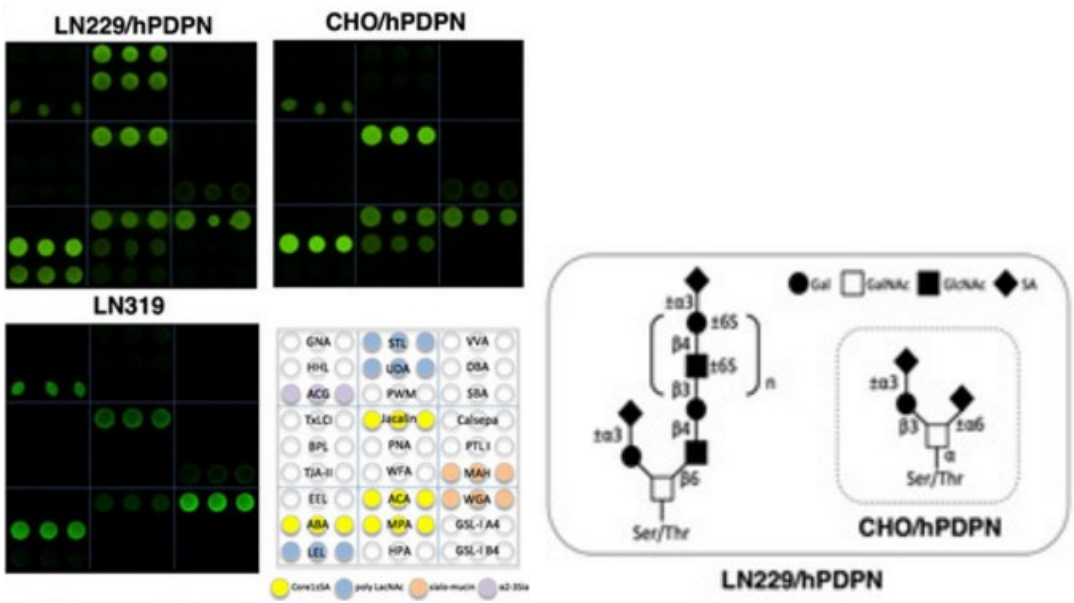
4. A Cancer-specific Monoclonal Antibody (CasMab)

Podoplanin(PDPN), a platelet aggregation-inducing mucin-like sialoglycoprotein, is highly expressed in many cancers and normal tissues. It was found from studies using Lectin Microarrays that podoplanin in cancer cells (LN229/hPDPN) has both polylactosamine and sialylated core1, whereas podoplanine in normal cells (CHO/hPDPN) has only sialylated core1. To develop novel anti-podplanin mAbs, Kato et. Al., immunized mice with LN227/hPDPN cells, which has cancer-type glycan patterns, and succeeded in developing such a CasMab, LpMab-2. This one would be a candidate for use in an antibody-drug conjugate.



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A Cancer-specific Monoclonal Antibody Recognizes the Aberrantly Glycosylated Podoplanin.

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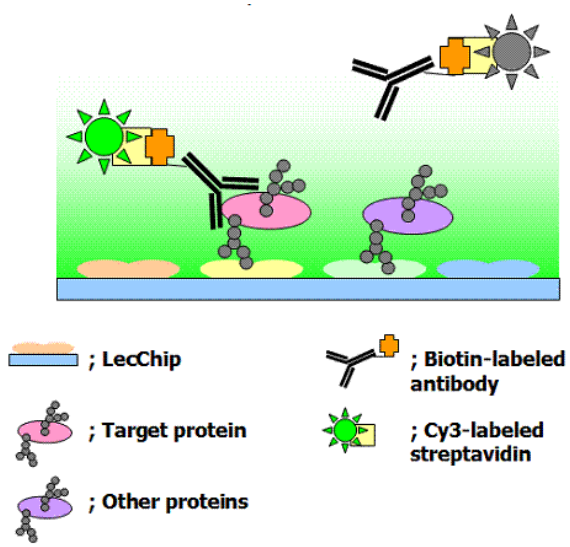
5. Biomarker Development and Antibody Overlay Lectin Microarray Method

Using an antibody overlay method with lectin microarray enables the investigation of glycans from a specific protein in a crude sample and to build "examination lists "effectively without detailed glycan structures.

For example, this method is used for investigation of Podoplanin which is a mucin-type sialoglycoprotein that acts as a platelet-aggregating factor in cancer cells. The investigators use biotinated NZ-1 which is a highly active anti-Podoplanin antibody and inhibits platelet aggregation induced by Podplanin. After the incubation of Podplanin sample on lectin microarray, biotinated NZ-1 is applied on it and its fluorescence is measured by Cy3 labeled streptavidin. From the analysis of lectin microarray, used Podoplanin sample expressed in Lec1、Lec2、Lec8, they point out that it has a structure of sialylated core 1. And from the examination of LN319 Podoplanin sample which express Podoplanin highly among 15 glioblastoma cell lines and induced platelet aggregation, the investigators point out that it has disialyl-T antigen, sialyl-T antigen and sialyl-Tn antigen.



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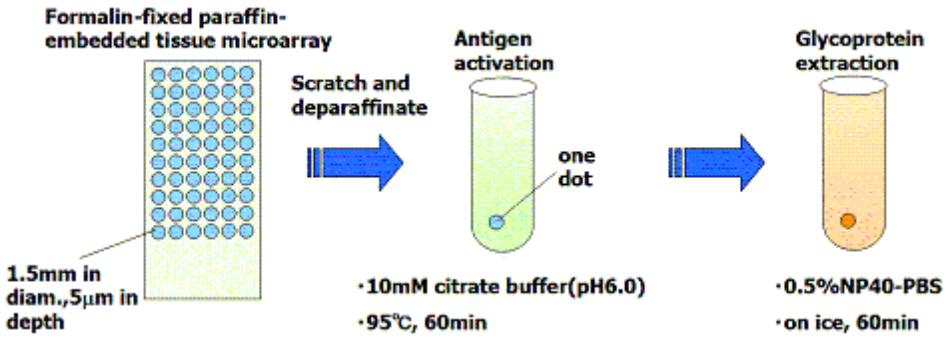
6. Glycan Profiling Analysis of Formalin-fixed tissue and New Biomarker Development

Glycan profiling analysis protocol of formalin-fixed tissue has also established. This method is very effective for new biomarker development since paraffin-embedded tissue samples are widely used in pathological analysis. For example, numerous differences were found in several lectins between normal colon tissues (n=12) and colon cancer tissues (n=28). Most notably, the data showed an increase P<0.0001 as a result by WFA, both of GradeI+II and GradeIII, and the correlativity with the results of histological stain by WFA was also confirmed. This ultra-sensitive method requires approximately **500 cells**. Antigen activation which is often performed in an immune tissue staining can increase the fluorescence labeling rate because formalin fixing makes cross-links loosen fully. Glycolipids are eliminated during the deparaffinizing process as well.



Applications for bile duct cancer, liver cancer, esophageal cancer, breast cancer, uterine body cancer and others are reported as the similar cases.

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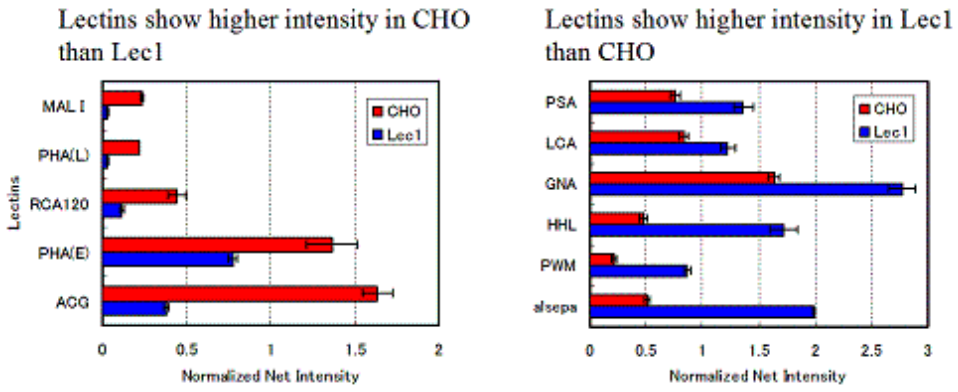
7. Glycan Profiling Analysis of Crude Samples

GlycoStation™ is very effective for differential analysis of crude samples such as cell lysate, cell extract (cell membrane, cytoplasmic compartment, etc.). In one instance, figures below show the results of glycan profiling analysis between membrane fraction of CHO and Lec1 mutant cells. As some information which is easily discovered through differential profiling, signals from branched complex-type N-glycan binders (PHA(L), PHA(E), ACG), □2,3-Sialic acid binder (MAL I), lactose binder (RCA120) drastically decrease in Lec1 mutant cells (shown in the graph of the lower left side). On the contrary, signals from high-mannose type N-glycan binders (GNA, HHL, PWM, Calsepa, PSA, LCA) increase in Lec1 mutant cells (shown in the graph of lower right side). These observations are quite reasonable, taking into consideration of the lack of glycosyltransferase GlcNAc-T1 in Lec1.



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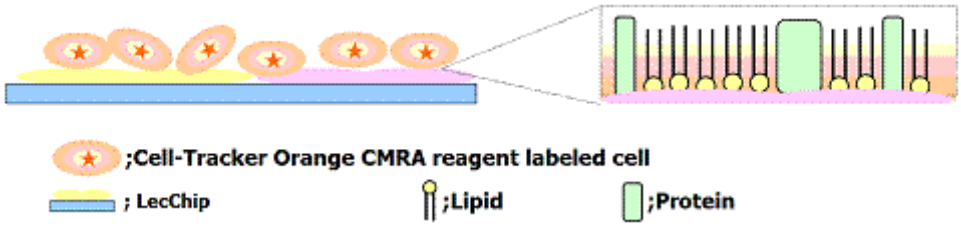
8. Cell Surface Glycome Profiling Analysis of Living Cells

It is well known that the cell surface glycome changes from species to species by differentiation stages and development of malignant variations. Though our standard protocol is based on a glycan profiling analysis using Cy3 labeled protein as a sample, it is also possible to investigate cell surface glycome of living cells using a metabolically-labeled sample by using a Cell-Tracker Orange CMRA regent. This method makes it possible to investigate the whole glycan structure includes glycolipid on cell surface as well as glycoprotein. We have gained particular results coincides very well with phenotype of glycosylation by differential profiling between CHO and its glycosylation-defective mutant cells, wild-type mouse's splenic cells and those of β1-3-N-acetylglucosaminyltransferaseII knockout mouse.

We have also found significantly increased expression of O-linked glycosylation among differentiated cells by differential profiling of cell surface glycome of K562. This method is the innovative protocol for profiling of mammalian cell surface glycome's variations, and applies to living cells.



Reference:

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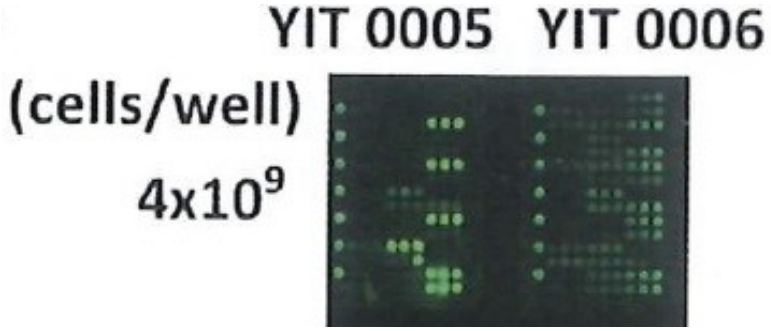
9. Intestinal Bacteria and Probiotics

It is well known that intestinal bacteria are deeply involved in human immune activity, health and longevity. There had been no method to investigate glycans of intestinal bacteria in an easy way, though it had been pointed out that they correlate deeply with the activation of intestinal immune system. However, lectin microarray enables us to profile glycome of intestinal bacteria surface quite simply and quickly by labeling its cell nuclei using SYTOX Orange. This is a joint R&D result between Yakult Central Institute for Microbiological Research and AIST, and now attracting so much attention as a powerful method promoting probiotics-related research and development.



Reference

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10. Glycan Profiling Analysis for Cytokine, Enzyme, Antibody Drug, etc. and Molecular Activity

As the most widely known example, medical benefits of antibody based drugs becomes different as much as 100 times in terms of its effect depends on whether there is modified Cure Fuc or not. Difference of glycan structure makes a great effect on molecular activity because not only IgG, but most protein is subject to glycan modification. Lectin microarray is the easiest system to profile glycoprotein.

References

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Pichia pastoris-produced mucin-type fusion proteins with multivalent O-glycan substitution as targeting molecules for mannose-specific receptors of the immune system.

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11. Transplant and Glycans

For example, □1-3Gal antigen becomes a major hurdle when you try to transplant grunter organ to human. Lectin microarray is also used to perform a simple test whether the glycan structure changes as expected, as in the case that you want suppress immune rejection response by using GalT-KO grunter.

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Survey of glycoantigens in cells from alpha1-3galactosyltransferase knockout pig using a lectin microarray.

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http://www.ncbi.nlm.nih.gov/pubmed/23549931

12. ATL and Glycans

Adult T-cell leukemia/lymphoma (ATL) is a refractory leukemia/lymphoma which symptoms begins 40 to 60 years after infection of Human T cell leukemia/lymphotropic virus type 1 (HTLV-1) and classified into four clinical subtypes (Smoldering type, Chronic type, Acute type, Lymphoma type). Advanced Acute type and Lymphoma type have an extremely bad prognosis and it's impossible to distinguish clinical subtypes by FCM, and it becomes a big problem when you consider prognosis of patients. It is reported that Lectin microarray is able to detect pathological differences of ATL cells well enough, and it shows that glycan profiling technology can be extremely pathologically useful for characterization of cells.

References

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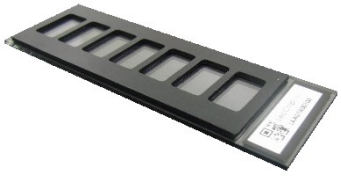
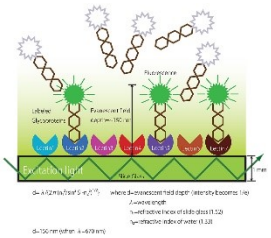
Hidekatsu Iha and Masao Yamada, "T-Cell Leukemia - Characteristics, Treatment and Prevention", ISBN 978-953-51-0996-9: 2013.DOI: 10.5772/55386.

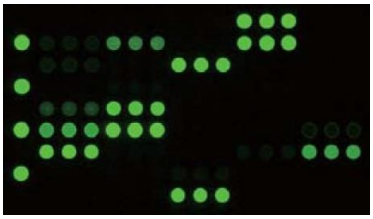
http://www.intechopen.com/books/t-cell-leukemia-characteristics-treatment-and-prevention/glycan-profiling-of-adult-t-cell-leukemia-atl-cells-with-the-high-resolution-lectin-microarrays

Further references: http://www.glycotechnica.com/english/application.html#reference

Please click on the corresponding text for more information on our **Glycan Analysis Testing Service** or on the commercially available **Glycan Profiling Technology**.







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