PRODUCT GUIDE

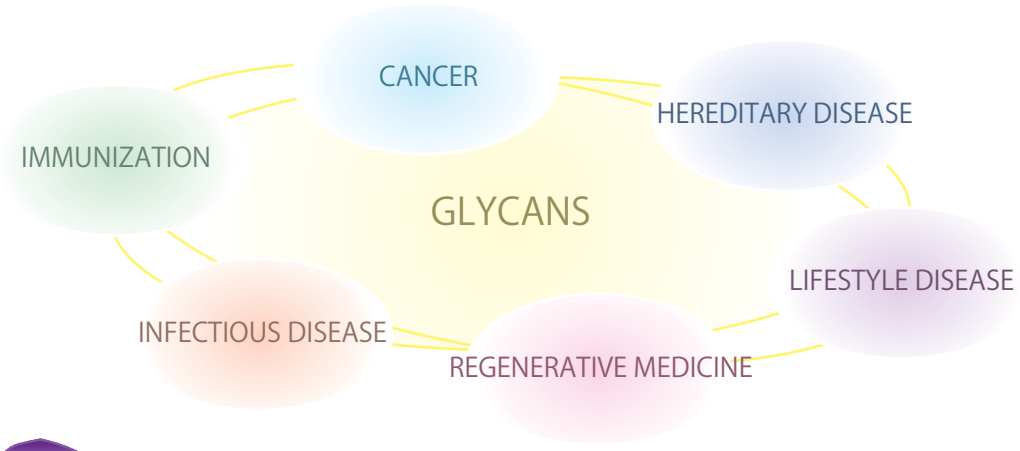
GlycoStation™

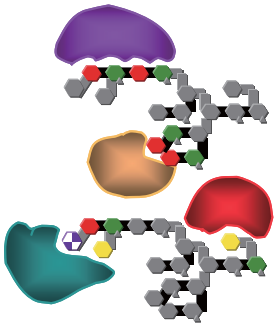
- Glycan Profiling System -

GlycoTechnica

Glycan Profiling System : GlycoStation™

Glycans play important roles in the field of life science. For instance, carbohydrate antigen plays very important roles in the following area as depicted below. As a research tool for biological and medical field, we have developed a new GlycoStation™ system for glycan structure profiling using lectin microarray and evanescent-field fluorescence excitation. This system is found to be of great use as a glycan structure profiling tool with rapid and easy operation. (joint development with National Institute of Advanced Industrial Science and Technology, AIST)

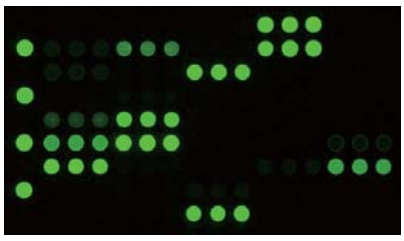




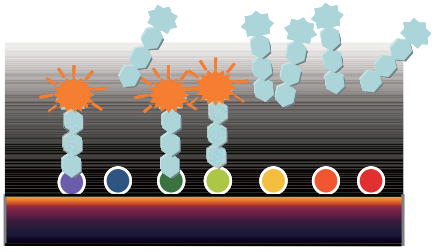
Glycan Binding Specificities of Lectins

The basis for this technology is glycan binding protein (i.e. lectin) arrayed chip. Carefully selected lectins from the point of view of glycan binding specificities and stabilities are immobilized on to slide glasses. Hence, it becomes possible to simultaneously detect affinity between various lectins with different binding capabilities and samples.
Using glycan protein sample of nano-gram order, it is possible to obtain the signal pattern corresponding to the glycans of the sample.

Moreover, in order to detect signals, we have developed a scanner which is capable of measuring liquid phase chips where a sample is applied, using evanescent-field fluorescent excitation. Evanescent-field is formed on the surface of low refractive index side when the incudent light is fulfilled with total reflection conditions. Its thickness is in the range of wave length of light, and the strength decreases exponentially with increasing distance from the surface. Owing to this real nature of evanescent-field, fluorescent labeled samples around slide surface are binded with lectin are excited at high efficiency but other fluorescent dyes are not excited at all. Applying this principle, it becomes possible to measure weak mutual molecular interaction such as lectin and glycans without washing process.
We are manufacturing and marketing this new technological product, glycan profiling system , GlycoStation™, as well as providing analysis service under contract.



An Example for LecChip Signal Pattern



Conceptual Diagram for Evanescent-Field Excitation Method

Chip

Lectin Microarray：LecChip™

LecChip™ ver. 1.0 contains 45 different lectins carefully selected out of 167 possible candidates considering binding specificity and stability. Lectins can be categorised to several groups depending on binding capabilities to glycans, for example, fucose binding lectin (AAL etc.), sialic acid binding lectin (SNA etc.), galactose binding lectin (ECA etc.), mannose binding lectin (GNA etc.), O-glycan binding lectin (Jacalin etc.), branching structure binding lectin and others. Using LecChip™ ver.1.0, it is now possible to measure binding between various lectins and samples and then to profile glycans contained in the samples. Each LecChip ver. 1.0 contains 7 independent wells and it is possible to measure 7 samples at the same time. Each of the 45 lectins of different types are placed in triplicate respectively.
The probing solution specifically blended for sample preparation is highly recommended in order to obtain better SN ratio of the glycan profiling patterns.

Lectin	45 species, 3 spots per each lectin
Well	7 wells on one chip, well volume = 100μl
CV Value	＜20%
Quality Guarantee Period	6 months
Storage Temperature	−20℃

LecChip™ ver.1.0 Product Specification

LecChip™ ver.1.0

Probing Solution

Scanner

Evanescent-Field Excitation Scanner：GlycoStation™ Reader 1200

GlycoStation™ Reader 1200 is a microarray scanner based on the principle of evanescent-field fluorescent excitation. It is now possible to measure very weak biomolecular interactions in the liquid phase without any washing process required for glycan profiling by lectin microarray. GlycoStation™ Reader 1200 is capable of scanning a chip in approximately two minutes.

Light source		Metal halide lamp: 350W [1]
Excitation wavelength		531nm(corresponding to Cy3) [2]
Function	Pixel Resolution	5μm
	Scanning Time	2min/chip (Exposure time: 133msec)
	Exposure Time	33 ～ 499msec
	Camera Gain	50 ～ 125
	Data Format	16bitTIFF, JPEG, Bitmap
	Indication of lamp used time	Indication every 500 hours
External Connection		USB x3, LAN(1000M) x1, VGA x1, Mouse, Keyboard
Input Voltage		AC100 ～ 240V
Power Consumption		Main unit: 650W, Light Source: 450W
Dimensions		Main unit: W440×D592×H585(mm) Light Source: W170×D340×H225(mm)
Weight		Main unit: 70kg, Light Source: 8.5kg

GlycoStation™ Reader 1200 Product Specification

GlycoStation™ Reader 1200

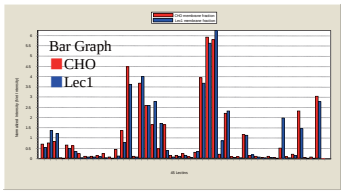
1) Lamp Life : approx. 1500 hours (60 % of the initial intensity)
2) Wavelength for measurement can be altered by changing the filter set (Excitation filter : max. 4 positions , fluorescent filter : max. 8 positions)

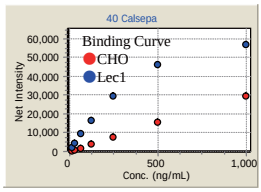
Software for Comparison and Analysis of Glycan Structure Profile : GlycoStation™ Tools

GlycoStation™ Tools ver.1.5 is a software for graphical display of glycan structure profile results. As a standard function, normalization of intensity, various graphical display, and exporting fanction of data for other softwares are included.

Using this, an example of a differential profiling of Lec1 mutant cells against CHO cells is shown on the right side. Using GlycoStation™ Tools, bar charts and concentration curves can be drawn immediately as shown in the diagram.

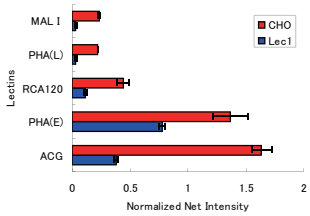
The diagrams below indicate typical signals of lectins whose difference is evident between two samples. The difference, in particular, in lectins recognizing N-glycans are remarkable, whereas it is small for lectins recognizing O-glycans. Signals from lectins recognizing complex type N-glycans (PHA(L), PHA (E). ACG), lectin recognizing α 2,3-Sia (MAL I), and lectins recognizing galactose (RCA 120) are seen decreasing in Lec1 mutant cells (see diagram below on the left). On the contrary, signals of lectins recognizing high -mannose type N-glycans (GNA, HHL, PWM, Calsepa, PSA, LCA) increase in Lec1 mutant cells (see diagram below on the right). These results coincide with expectation for Lec1 lacking GlcNac-transferase I (GlcNAc-T1) (see Y. Ebe et al., J. Biochem, 139, p.323 (2006))



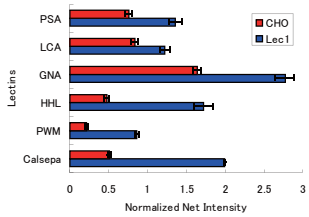


Measuring Method using GlycoStation™ System

Lectin showing high signals in CHO

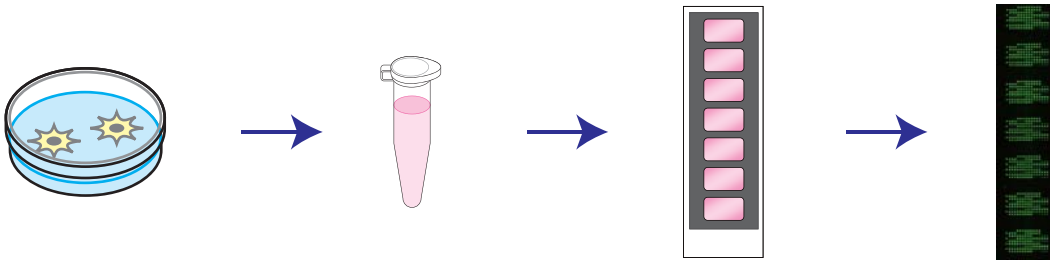


Lectin showing high signals in Lec1



Measuring Method using GlycoStation™ System

GlycoStation™ System enables you to measure various samples ranging not only purified proteins but also crude samples such as cells and tissues. Their measuring methods also cover various assays, such as detection of fluorescent labeling directly from proteins, sand-witch assay by antibodies, and measurement of glycome on the living cells. One example is shown below for measuring glycan profiling for cultivated cells. Please refer to our Home Page for other applications and protocols in detail.



Pre-treatment

-In case of cultivated cells, prepare either laysate or fractionation

Fluorescent labeling

- Quantitation of proteins in samples using MicroBCA method
- Cy3 labeling
-- Exclusion of unreacted Cy3 using gel filtration

Sample reaction

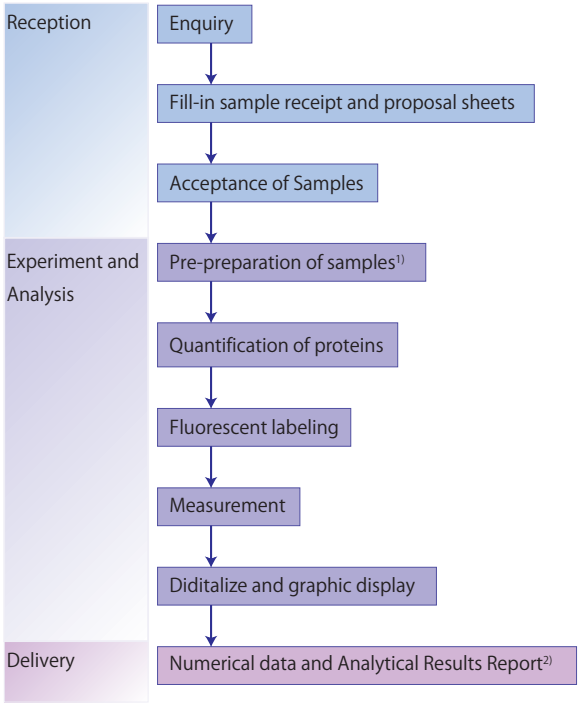
- Dilute fluorescent labeled sample with Probing Solution
- Add samples to chips.

Measurement and Analysis

- Measurement and Analysis are made using GlycoStation™ 1200 and GlycoStation™ Tools.

Contract Service for Glycan Analysis

GlycoTechnica undertakes glycan analysis contract service using GlycoStation™ system.



1) Pre-treatment for Samples：

Optional service will be provided for samples of buffer exchange to PBS and concentration and cell section etc.

2) Analysis Result Report：

Number of sample comparison in the analysis result report is based on the comparison of samples against the control sample. In case of multiple number of control samples and multiple comparison we reserve our right to discuss the service costs separately.

【Acceptance of Samples】

This service is based on differential analysis, and hence please provide more than 2 samples, namely, control and samples to be compared. Preparation of samples is detailed below.

Protein solutions

- Prepare 2 samples each consisting of protein concentration more than 50 μg/ml and sample amount more than 20 μL. Use PBS as buffer.

Cultivated Cells

- in case of cultivated cells, prepare 2 samples each for cell number more than 10 to the sixth, wash well with PBS and make pellets after cultivated area is removed.

Please note above are typical conditions and it may be possible to measure less amount of samples than above and on the other hand sometimes it may not be possible to measure even using above conditions. Moreover, for samples other than above conditions, it would be different depending on sample conditions and treatment methods. Please refer to us for details.

【Sample Dispatch】

Human samples must be sent anonymously according to informed consent. Please also confirm the samples are free from infectious viruses such as HIV, HCV, etc. After proposal is accepted, samples shall be sent in frozen state or cold storage.

【Experiment and Analysis】

Experiment and analysis are performed based on our protocol.

【Delivered Sample】

Data for delivered samples contain following information :

- Numerical data … Excel File for LecChip raw data are stored in CD-R.
- Analysis Results Report … this report contains explanation of structure comparison of glycans between samples based on lectin glycan recognition capability.

Publications

"Evanescent-field fluorescence-assisted lectin microarray: a new strategy for glycan profiling", Atsushi Kuno, Noboru Uchiyama, Shiori Koseki-Kuno, Youji Ebe, Seigo Takashima, Masao Yamada and Jun Hirabayashi, *Nature Methods*, **2**, 851-856 (2005).

"Application of Lectin Microarray to Crude Samples: Differential Glycan Profiling of Lec Mutants", Youji Ebe, Atsushi Kuno, Noboru Uchiyama, Shiori Koseki-Kuno, Masao Yamada, Takashi Sato, Hisashi Narimatsu and Jun Hirabayashi, *J Biochem*, **139**, 323-327 (2006).

"The Era of Glycan Profiling has come: Glycomics's Infinite Potential and Applications to Healthcare", Masao Yamada, *GOR*, **9**, 16-18 (2007).

"A novel strategy for mammalian cell surface glycome profiling using lectin microarray", Hiroaki Tateno, Noboru Uchiyama, Atsushi Kuno, Akira Togayachi, Takashi Sato, Hisashi Narimatsu, and Jun Hirabayashi, *Glycobiology*, **17**, 1138-1146 (2007).

"Development of an all-in-one technology for glycan profiling targeting formalin-embedded tissue sections", Atsushi Matsuda, Atsushi Kuno, Hiroyasu Ishida, Toru Kawamoto, Jun-ichi Shoda and Jun Hirabayashi, *Biochemical and Biophysical Research Communications*, **370**, 259-263 (2008).

"Development of a data-mining system for differential profiling of cell glycoproteins based on lectin microarray", Atsushi Kuno, Yoko Itakura, Masashi Toyoda, Yoriko Takahashi, Masao Yamada, Akihiro Umezawa and Jun Hirabayashi, *Journal of Proteomics & Bioinformatics*, **1**, 68-72 (2008).

"Optimization of evanescent-field fluorescense-assisted lectin microarray for high-sensitivity detection of monovalent oligozaccharides and glycoproteins", Noboru Uchiyama, Atsushi Kuno, Hiroaki Tateno, Yoshiko Kubo, Mamoru Mizuno, Midori Noguchi and Jun Hirabayashi, *Proteomics*, **8**, 3042-3050 (2008).

"Forcussed Differential Glycan Analysis with the Platform Antibody-assisted Lectin Profiling for Glycan-related Biomarker Verification", Atsushi Kuno, Yukinari Kato, Atsushi Matsuda, Mika Kato Kaneko, Hiromi Ito, Koh Amano, Yasunori Chiba, Hisashi Narimatsu, and Jun Hirabayashi, *Molecular & Cellular Proteomics*, **8**, 99-108 (2009).

MICROARRAY METHODS AND PROTOCOLS, Robert S. Matso, CRC Press Taylor & Francis Group (Chapter9：Lectin Microarrays, Masao Yamada) (2009).

GlycoTechnica

Iinquiry TEL：+81-45-913-5803 FAX：+81-45-511-8570 E-mail：info@glycotechnica.com URL：http://www.glycotechnica.com/english