Specification for Glycan Profiling Analysis Service

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1．GlycoStation Reader™ System

1）System Constitution

GlycoStation Reader™ System consists of LecChip™, GlycoStation Reader™1200 and analysis software, ToolsPro

LecChip™

GlycoStation Reader™ 1200

2）Profiling Basis

① 45 different lectins

Lectin is a collective term of protein (excludes antibody, enzyme) which specifically recognizes glycans. Epitope structure binded glycans is different according to the lectins, therefore profiling the lectin's binding pattern (profiling pattern) enables you to identify the structural features.

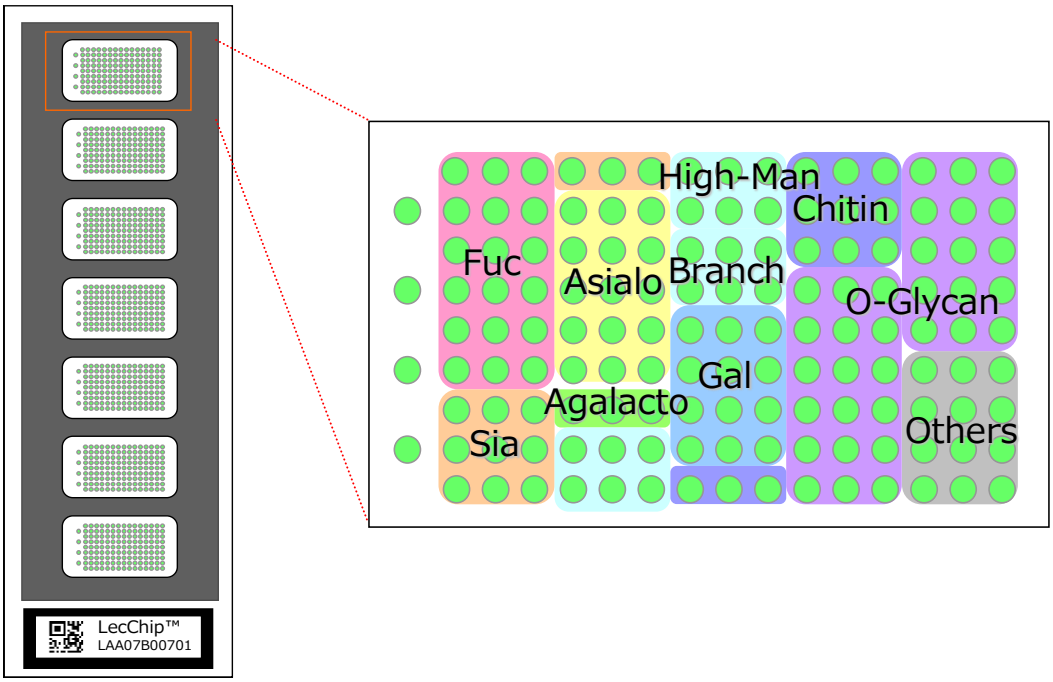
LecChip™ is a fixed slide glass that is spotted carefully-selected 45 kinds of lectins (refer to the next list). You can see glycans that are specifically recognized by 45 kinds of lectins in the next simply-sorted list. Please note that the list doesn't show all of the glycan bindings by lectin.

		Edited by M. Yamada, 8/21/2007
Lectin No.	Lectin	Reported specificity
1	LTL	Fuca1-3(Galb1-4)GlcNAc, Fuca1-2Galb1-4GlcNAc
2	PSA	Fuca1-6GlcNAc, a-D-Glc, a-D-Man
3	LCA	Fuca1-6GlcNAc, a-D-Glc, a-D-Man
4	UEA-I	Fuca1-2Galb1-4GlcNAc
5	AOL	Fuca1-6GlcNAc (core fucose)
6	AAL	Fuca1-6GlcNAc, Fuca1-3(Galb1-4)GlcNAc
7	MAL	Siaa2-3Galb1-4GlcNAc
8	SNA	Siaa2-6Gal/GalNAc
9	SSA	Siaa2-6Gal/GalNAc
10	TJA-I	Siaa2-6Gal/GalNAc
11	PHAL	tri/tetra-antennary complex-type N-glycan
12	ECA	Galb1-4GlcNAc
13	RCA120	Galb1-4GlcNAc
14	PHAE	bi-antennary complex-type N-glycan with outer Gal and bisecting GlcNAc
15	DSA	(GlcNAcb1-4)n, Galb1-4GlcNAc
16	GSL-II	agalactosylated tri/tetra antennary glycans, GlcNAc
17	NPA	High-Mannose, Mana1-6Man
18	ConA	High-Mannose, Mana1-6(Mana1-3)Man
19	GNA	High-Mannose, Mana1-3Man
20	HHL	High-Mannose, Mana1-3Man, Mana1-6Man
21	ACG	Siaa2-3Galb1-4GlcNAc
22	TxLCI	Mana1-3(Mana1-6)Man, bi- and tri-antennary complex-type N-glycan, GalNAc
23	BPL	Galb1-3GalNAc, GalNAc
24	TJA-II	Fuca1-2Galb1-> or GalNAcb1-> groups at their nonreducing terminals
25	EEL	blood group B antigen, Gala1-3Gal
26	ABA	Galb1-3GalNAc
27	LEL	GlcNAc trimers/tetramers
28	STL	GlcNAc oligomers, oligosaccharide containing GlcNAc and MurNAc
29	UDA	GlcNAcb1-4GlcNAc, Mixture of Man5 to Man9
30	PWM	(GlcNAcb1-4)n
31	Jacalin	Galb1-3GalNAc, GalNAc
32	PNA	Galb1-3GalNAc
33	WFA	GalNAcb1-4GlcNAc, Galb1-3(-6)GalNAc
34	ACA	Galb1-3GalNAc
35	MPA	Galb1-3GalNAc, GalNAc
36	HPA	a-linked terminal GalNAc
37	VVA	a-linked terminal GalNAc, GalNAca1-3Gal
38	DBA	blood group A antigen, GalNAca1-3GalNAc
39	SBA	a- or b-linked termincal GalNAc, GalNAca1-3Gal
40	Calsepa	Mannose, Maltose
41	PTL-I	a-linked terminal GalNAc
42	MAH	Siaa2-3Galb1-3(Siaa2-6)GalNAc
43	WGA	chitin oligomers, Sia
44	GSL-I A4	a-linked GalNAc
45	GSL-I B4	a-linked Gal
Remarks) These data were collected from lectin vendors and reports found by internet searches.		

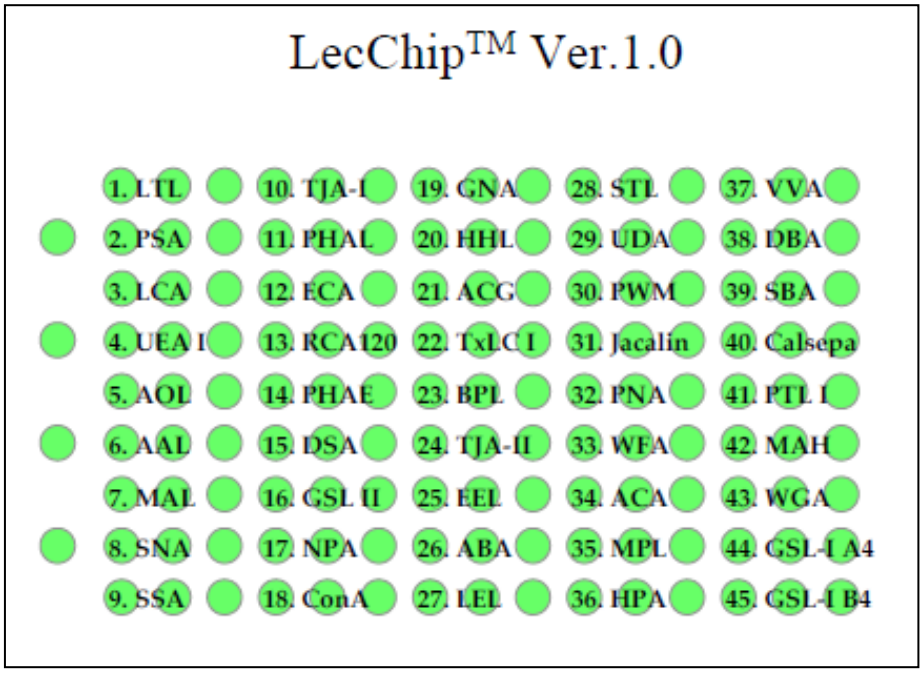
② LecChip™

LecChip's glass is covered by rubber and forms 7 wells as shown in the figure under left. Each well's capacity is 100μL.

The same format, carefully-selected 45 kinds of lectins are spotted in triplicate as shown in the extended figure under right.



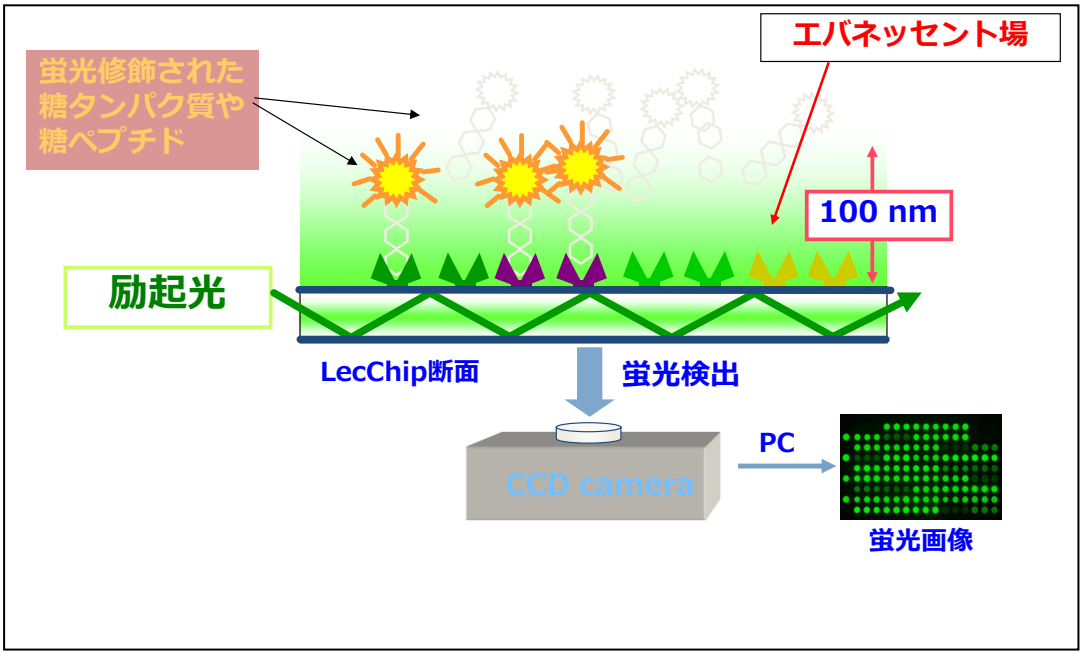
Detailed array of lectins is as described below.



③ GlycoStation Reader™ 1200

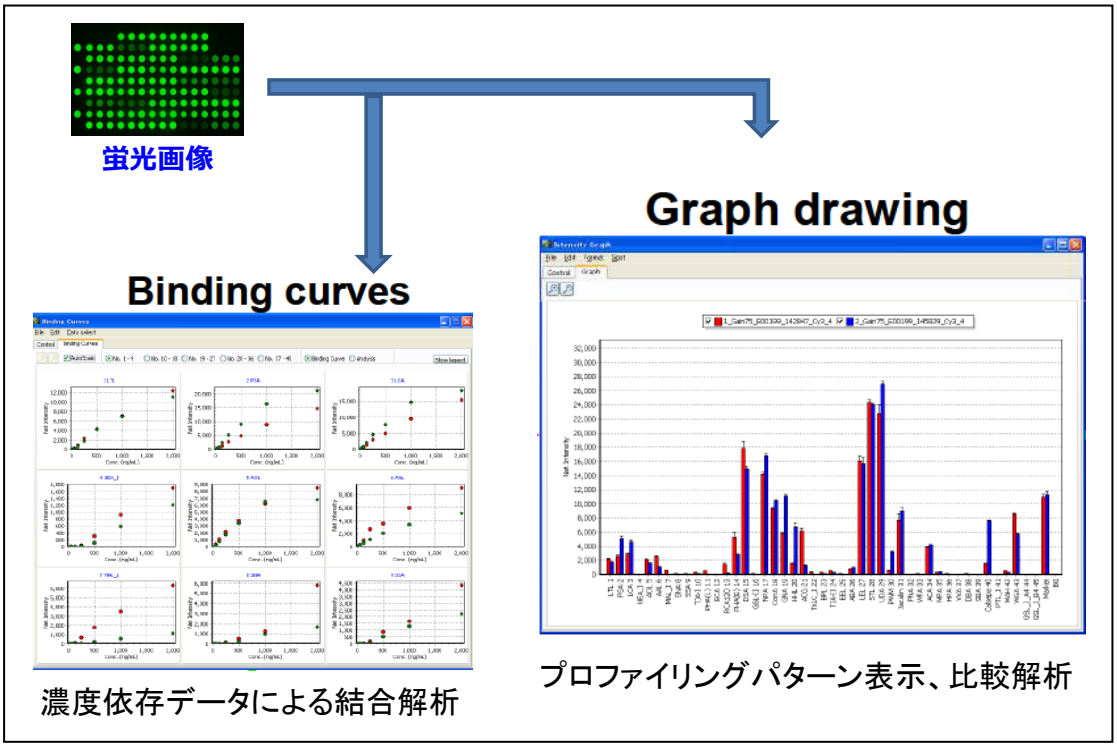
GlycoStation Reader 1200 is created as a dedicated profiler (i.e., scanner) for LecChip™

Since binding strength between glycan and lectin is relatively weak, washing process after their binding reaction as you do on a DNA array or antibody array brakes off the week bindings. GlycoStation Reader 1200 can screen and detect only the light of fluorescently-labeled glycan binding lectin fixed on the slide glass in high S/N ratio without washing process by making evanescent-wave formed under the heights of 200nm from the substrate interface into excitation light of fluorescence.



④ ToolsPro

GlycoStation™ToolsPro is software that analyzes fluorescence images obtained from GlycoStation Reader 1200 and transforms them into Binding Curves or Comparative Glycan Profiling Images as described below.



２．Detailed Experimental Protocol

Glycan Profiling Analysis

１．Analysis Object

GlycoStation Reader™ System can analyze a wide variety of sample forms such as cultivated cells, biopsy tissue sections, paraffin fixed tissue sections, serum, urine, culture supernatant fluid and milk. The pre-treatment procedures are different for different types of samples. Basic protocol, itself is very easy to conduct and the main procedures are protein quantitation and Cy-3 labeling.

２．Analysis procedure (in the case of biopsy sample)

２－１．Add 1/100 of Protease Inhibitor Cocktail, EDTA-Free(×100) to PBS-Tx and mix.

２－２．Put biopsy sample into sample tube for homogenization, add 200uLof ２－１ solution to biopsy tissue and homogenize it for 30~60 sec.

２－３．Add 300uL of ２－１ solution to ２－２ sample and sonicate it for 1 min.

２－４．Centrifuge it at 4℃, 14000×g for 5 min and recovery supernatant(soluble fraction).

２－５．Dilute samples to 50ug/mL by adding PBS based on the result of the Micro-BCA Protein Assay.

２－６．Mix 20uL of ２－５ sample and Cy3 Mono-Reactive dye 100ug Labeling and incubate it for 1 hour at R.T. in the dark.

２－７．Centrifuge desalinated column at 4℃, 1500×g for 1 min.

２－８．Apply 300uL of TBS to ２－７ desalinated column and centrifuge it at 4℃, 1500×g for 1 min.

２－９．Repeat ２－８ twice.

２－１０．Apply total amount of ２－６ sample and 25uL of TBS to ２－９ desalinated column, centrifuge it at 4℃, 1500×g for 2 min and remove unreacted Cy3.

２－１１．Dilute the sample to 7 steps of dilution series in increments of 1/2 from 2ug/mL by adding Probing Solution.

２－１２．Melt LecChip from －20℃ and wash the surface of inside wells 3 times with Probing Solution.

２－１３．Apply diluted ２－１１ sample to LecChip and incubate it at 20℃ agitating with a shaker over night.

２－１４．Scan the Lecchip with GlycoStation Reader 1200 and digitalize it's fluorescence image with GlycoStation Tools Pro.

３．Analysis procedure（in the case of cell pellet：Whole Cell Lysate）

３－１．Mix PBS and TritonX-100, dilute PBS-T(1%TritonX-100), add PBS-T=1mL to the cell pellet and suspend with pipette. Sonicate it for 1 min for cell breakage and centrifuge it at 4℃, 14000×g for 5 min and recovery supernatant.

３－２．Dilute samples to 50ug/mL by adding PBS based on the result of the Micro-BCA Protein Assay.

３－３. Mix 20uL of ３－１ sample and Cy3 Mono-Reactive dye 100ug Labeling and incubate it for 1 hour at R.T. in the dark.

３－４. Centrifuge desalinated column at 4℃, 1500×g for 1 min.

３－５. Apply 300uL of TBS to ３－４ desalinated column and centrifuge it at 4℃, 1500×g for 1 min.

３－６. Repeat ３－５ twice.

３－７. Apply total amount of ３－３ sample and 25uL of TBS to ３－６ desalinated column, centrifuge it at 4℃, 1500×g for 2 min and remove unreacted Cy3.

３－８. Dilute the sample to 7 steps of dilution series in increments of 1/2 from 2ug/mL by adding Probing Solution.

３－９. Melt LecChip from －20℃ and wash the surface of inside wells 3 times with Probing Solution.

３－１０. Apply diluted ３－８ sample to LecChip and incubate it at 20℃ agitating with a shaker over night.

３－１１. Scan the Lecchip with GlycoStation Reader 1200 and digitalize it's fluorescence image with GlycoStation Tools Pro.

The above protocol is an example of analysis procedure for Whole Cell Lysate, but it's also possible to profile glycans from membrane protein alone extracted with fractionation kit.

３. Delivery

１. Basic Analysis Plan：

Data about combining specificity between Lectin and glycan, Numerical Data (Excel File)

２. Differential Analysis Report Plan：

In addition to the above 1.data, report about the results of Differential Glycan Profiling Analysis (Clustering Analysis, ROC Analysis, on request) which contains explanation of structural comparison of glycans between samples.

GlycoTechnica

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