RayBio® Human Glycosylation Antibody Array L-4

For the simultaneous detection of the glycosylation protein profile of 500 human proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

User Manual (Revised Nov. 4th, 2020)

Human Glycosylation Array L-4
Cat# GAH-GCM-L-4-4 (4 Sample Kit)
Cat# GAH-GCM-L-4-8 (8 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
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Your Provider of Excellent Protein Array Systems and Services

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I. Introduction

Glycosylation is the addition of sugar molecules onto cellular proteins, lipids, and polysaccharides that results from a co- and post-translational modification. Recent appreciation of glycosylation changes on the cell surface have been linked to numerous cellular diseases, most notably in several cancers. Understanding the changes that occur in the cellular process of molecule glycosylation can unveil new biomarkers of disease or pave the way for new drug targets.

Recent technological advances by RayBiotech have enabled the largest commercially available glycosylation antibody array to date. With the Human Glycosylation Array L-4, researchers can now efficiently obtain glycosylation profiles in their samples. This assay allows the simultaneous detection of glycosylation profiles of L-4 human proteins, including, but not limited to, cytokines, chemokines, adipokines, growth factors, angiogenic factors, proteases, soluble receptors, soluble adhesion molecules in cell culture supernatants, serum, plasma and other samples types. Using a set of 5 lectins that recognize a broad array of glycosylation moieties on the surface of proteins, this assay allows researchers to identify glycosylation changes on the surface of these proteins directly.

How it Works

Capture antibodies for the L-4 proteins are printed onto glass slides and the glycans on these capture antibodies are removed. The glass slide arrays come pre-blocked and are ready to be incubated with samples. After incubation with samples and washing to remove unbound proteins, five unique biotin-labeled lectins are incubated with the array. These lectins bind their respective glycan moiteies on the captured proteins present on the glass surface. Streptavidin-conjugated fluorescent dye (Cy3 equivalent) is then added to the array which recognizes the biotin attached to any bound lectin molecule. Finally, the glass slide is dried and laser fluorescence scanning is used to visualize the signals. These signals are then compared to the array map to identify glycosylated proteins present in the samples.

II. Materials Provided

A. Storage Recommendations

Upon receipt, the kit should be stored at -20°C until needed. Use within 6 months from the date of shipment is recommended. After initial use, remaining reagents should be stored at 4°C and may be stored for up to 3 months. Unused glass slides should be kept at -20 °C and repeated freeze-thaw cycles should be avoided (slides may be stored for 6 months).

ITEM	Description	4 Sample Kit (GAH-GCM-L-4-4)	8 Sample Kit (GAH-GCM-L-4-8)
A	Human Glycosylation Array L-4 Glass Slide*	1 pre-coated slide	2 pre-coated slides
B	Dilution Buffer	1 bottle (8 mL)	2 bottles (8 mL/ea)
D	20X Wash Buffer 1 Concentrate	1 vial (30 ml)	2 vials (30 ml/ea)
E	20X Wash Buffer 2 Concentrate	1 vial (30 ml)	2 vials (30 ml/ea)
F	Cy3-Conjugated Streptavidin**	1 vial	2 vials
G	Biotin-labeled Lectin Mixture***	2 vials	4 vials
H	Adhesive Plastic Strips	1	2
M	30 mL Centrifuge Tube	1 tube	1 tube

*Each slide contains 4 identical subarrays
**HiLyte Plus™ 555
*** See page 13 for lectin mixture composition

B. Additional Materials Required

- ddH_2O
- Small plastic or glass containers
- Orbital shaker or oscillating rocker
- 1 ml tube
- Pipettors, pipette tips and other common lab consumables
- Laser scanner for fluorescence detection (list available online)
- Aluminum foil

III. Overview and General Considerations

A. Preparation and Storage of Samples

1) Preparation of Cell Culture Supernatants

1. Seed cells at a density of $1x10^6$ cells in 100 mm tissue culture dishes.*
2. Culture cells in complete culture medium for ~24–48 hours. **
3. Replenish with serum-free or low-serum medium such as 0.2% FCS/FBS serum, and then incubate cells again for ~48 hours. ** If high serum medium such as 10% FCS/FBS is used, high background can occur on glass slide arrays due to serum interactions with the slide surface.
4. To collect supernatants, centrifuge at 1,000 g for 10 min and store as ≤1 ml aliquots at -80°C until needed.
5. Measure the total wet weight of cultured cells in the pellet and/or culture dish. You may then normalize between arrays by dividing fluorescent signals by total cell mass (i.e., express results as the relative amount of protein expressed/mg total cell mass). Or you can normalize between arrays by determining cell lysate concentration using a total protein assay (BCA Protein Assay Kit, Pierce, Prod #: 23227).

**The density of cells per dish used is dependent on the cell type. More or less cells may be required.*

***Optimal culture time may vary and will depend on the cell line, treatment conditions and other factors.*

2) Extracting Protein from Cells

1. Centrifuge Cells:

    a. Adherent Cells:

        i. Remove supernatant from cell culture and wash cells gently twice with cold 1X PBS taking care not to disturb cell layer.

        ii. Add enough cold 1X PBS to cover cell layer and use cell scraper to detach cells. Proceed to 1b. Cells in Suspension.

    b. Cells in Suspension: Pellet the cells by centrifugation using a microcentrifuge at 1500 rpm for 10 min.

2. Make sure to remove any remaining PBS before adding cell lysis buffer. Solubilize the cells at $2x10^7$ cells/ml lysis buffer.

Note: We recommend RayBio® 2X Cell Lysis Buffer (Cat# AA-LYS) or another lysis buffer suitable for immunoprecipitations. We also recommend supplementing the buffer with a protease inhibitor cocktail prior to homogenization.

3. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 13,000 rpm for 10 min at 2-8 °C.

Note: If the lysates appear to be cloudy, transfer the lysates to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the lysates are still not clear, store them at -20°C for 20 minutes. Remove from the freezer and immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

4. Transfer lysates to a clean tube. Determine cell lysate concentrations using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227). Aliquot the lysates and store at -80°C.

3) Extracting Protein from Crude Tissue

1. Transfer approximately 100 mg crude tissue into a tube with 1 ml lysis buffer.
2. Homogenize the tissue according to homogenizer manufacturer instructions.
3. Transfer extracts to microcentrifuge tubes and centrifuge for 20 min at 13,000 rpm (4°C).

Note: If the supernatant appears to be cloudy, transfer the supernatants to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the supernatant is still not clear, store the lysate at -20°C for 20 minutes. Remove from the freezer, immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

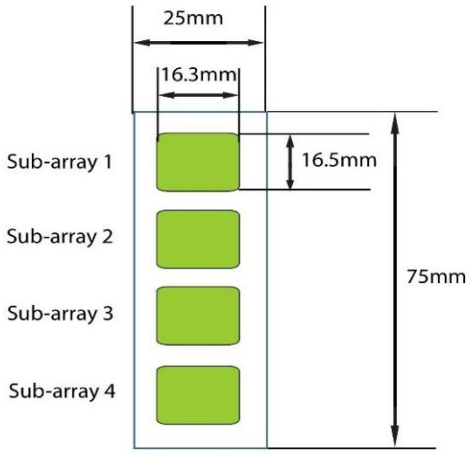
4. Transfer supernatant to a clean tube and store at -80°C.

B. Handling the Glass Slides

- The microarray slides are delicate. Please do not touch the array surface with pipette tips, forceps or your fingers. Hold the slides by the edges only.
- Handle the slides with powder-free gloves and in a clean environment.
- Do not remove the glass slide from the chamber assembly until step 14 on page 10, and take great care not to break the glass slide when doing so.
- Remove reagents/sample by gently applying suction with a pipette to corners of each chamber. Do not touch the printed area of the array, only the sides as seen in image below.

C. Layout of Human Glycosylation Array L-4 Glass Slide

Four identical sub-arrays on one slide



4 printed sub-arrays per glass chip

D. Incubations and Washes

- During incubations and washes, cover incubation chamber with a Plastic Adhesive Strip (Item H) to prevent evaporation, particularly those incubations lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and be sure to remove all bubbles from the sub-array surface.
- Perform all incubation and wash steps with a gentle rocking motion (~0.5 to 1 cycle/sec).
- Wash steps in Wash Buffer II and all incubation steps may be performed overnight at 4°C, with gentle rocking.
- Avoid cross-contamination of samples to neighboring wells. To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Slide Assembly to decant, and aspirate the remaining liquid.

- Unlike most Cy3 fluors, the HiLyte Plus™ 555 used in this kit is very stable at RT and resistant to photobleaching on the hybridized glass slides. However, please protect glass slides from strong direct light and temperatures above RT.

IV. Protocol

A. Drying of the Glass Slide

1. Remove the package containing the assembled glass slide (Item A) from the freezer. Place unopened package on the bench top for approx. 15 min, and allow the assembled glass slide to equilibrate to room temperature (RT).

2. Open package, and take the assembled glass slide out of the sleeve (do not disassemble the glass slide from the chamber assembly). Place glass slide assembly in laminar flow hood or similar clean environment for 1-2 hours at RT.

Note: Protect the slide from dust or other contaminants.

B. Incubations

Note: Glass slide should be completely dry before continuing with this procedure.

3. Immediately prior to sample incubation, spin samples for 5 min at 10,000 rpm to remove any particulates or precipitates. Dilute samples with 1X Dilution Buffer (Item B). Recommended dilution of samples with Blocking Buffer is 2-10 fold for serum/plasma and cell culture supernatants or body fluid, 100-500ug/ml (total protein concentration) for cell/tissue lysate.

Note: Optimal sample dilution factor will depend on the abundance of target proteins. If the background or antigen-specific antibody signals are too strong, the sample can be diluted further in subsequent experiments. If the signal is too weak, more concentrated samples can be used.

4. Add 400 µl of diluted samples into appropriate wells. Remove any bubbles on array surfaces. Incubate arrays with gentle rocking for 2 hours at RT or overnight at 4°C.

Note: Avoid sample flow through into neighboring wells.

5. Dilute 20X Wash Buffer I Concentrate (Item D) 20-fold with ddH_2O. Decant the samples from each well, and wash 3 times with 800 µl of 1X Wash Buffer I at RT with gentle rocking for 5 min per wash.

6. Obtain a clean container (e.g., pipette tip box or slide-staining jar), place the assembled glass slide into the container with enough volume of 1X Wash Buffer I to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking for 10 min per wash.

7. Dilute 20X Wash Buffer II Concentrate (Item E) 20-fold with ddH_2O. Decant the Wash Buffer I from Step 6. Place the assembled glass slide into the container with enough volume of 1X Wash Buffer II to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking for 5 min per wash.

8. Prepare Biotin-labeled Lectin Mixture (Item G): Add 1 mL 1x Dilution Buffer directly into the tube Item G. Pipette up and down gently to mix and then pipette 400 µL into each well on the array slide.

9. Incubate slide at RT with gentle rocking for 45-50 minutes.

10. Repeat washing steps from Step 5 to Step 7.

11. Prepare 1X Cy3-Conjugated Streptavidin:

    a. Briefly spin down tube containing the Cy3-Conjugated Streptavidin (Item F) immediately before use.

    b. Add 1000 µl of Dilution Buffer (Item B) into the tube to prepare a concentrated Cy3-Conjugated Streptavidin stock solution. Pipette up and down to mix gently (do not store the stock solution for later use).

    c. To prepare 1X Cy3-Conjugated Streptavidin, add 400 µl of the concentrated Cy3-Conjugated Streptavidin stock solution into a tube with 1600 µl of Dilution Buffer (Item B). Mix gently.

12. Carefully remove Assembled Glass Slide from container. Remove all of Wash Buffer II from the wells. Add 400 µl of 1X Cy3-Conjugated Streptavidin to each sub-array. Cover the incubation chamber with the plastic adhesive strips.

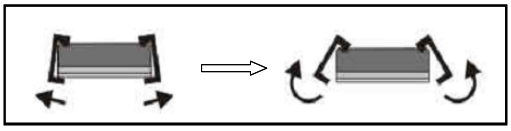
Note: Avoid exposure to light in Steps 13-20 by covering the Glass Slide Assembly with aluminum foil or incubate in dark room.

13. Incubate with Cy3-Conjugated Streptavidin at RT for 2 hours with gentle rocking.

***Note: Incubation may be done overnight at 4°C* with gentle rocking.**

14. Decant the solution and disassemble the glass slide from the incubation frame and chamber. Disassemble the device by pushing clips outward from the side, as shown below. Carefully remove the glass slide from the gasket.

Note: Be careful not to touch the printed surface of the glass slide, which is on the same side as the barcode.



15. Gently place the glass slide into the 30 ml Centrifuge Tube (Item I). Add enough 1X Wash Buffer I to cover the entire glass slide. Wash with gentle rocking for 10 min. Remove Wash Buffer I. Repeat 2 times for a total of 3 washes.

16. Repeat step 15 with 1X Wash Buffer II. Repeat one time with Wash Buffer II, for a total of two washes for 5 min per wash.

17. Finally, wash the glass slide with 30 ml of ddH_2O for 5 min with gentle rocking. Remove glass slide and decant water from Centrifuge Tube.

18. Remove water droplets from slide by applying suction gently with a pipette tip.

Note: Be careful not to touch the array portions of the slide with your pipette tip, only touch the sides of the slide.

C. Fluorescence Detection

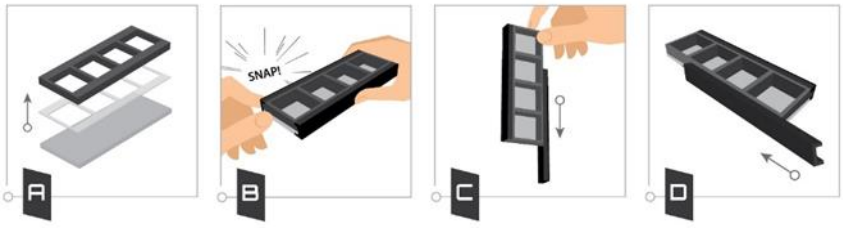
19. Allow glass chip to dry in a laminar flow hood protected from light for 20 minutes or until slide is completely dry. Place chip under an aluminum foil tent to protect it from light. Make sure the slides are absolutely dry before scanning or storage.

20. You may proceed immediately to scanning, or you may store the slide at -20 °C in the Centrifuge Tube provided or at RT and to scan at a later time.

Note: ***<u>Please protect glass slides from temperatures above RT and store them in the dark protected from light</u>. Do not expose glass slide to strong light, such as sunlight or a UV lamp.***

Note: ***If you need to repeat any of the incubations after finishing the experiment, you must first re-assemble the glass slide into the incubation chamber by following step as shown in the figures below. To avoid breaking the printed glass slide, you may first want to practice assembling the device with a blank glass slide.***

1. *Apply slide to incubation chamber barcode facing upward as in image A (below).*
2. *Gently snap one edge of a snap-on side as shown in image B.*
3. *Gently press other of side against lab bench and push in lengthwise direction (image C).*
4. *Repeat with the other side (image D)*



V. Antibody Array Map

RayBio® Human Glycosylation Antibody Array L-4 Map

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	P 1a	P 1a	P 2a	P 2a	P 3a	P 3a	neg	neg	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12	13	13	14	14	15	15
2	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26	27	27	28	28	29	29	30	30
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35	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	positi	posit	posit	posit	posit	positi

Biotin-Labeled Lectin Mixture

	Lectin Name	Sugar specificity
1	Concanavalin A	αMan, αGlc
2	Dolichos Biflorus Agglutinin	αGalNAc
3	Peanut Agglutinin	Galβ3GalNAc
4	Ulex Europaeus Lectin 1	αFuc
5	Wheat Germ Agglutinin	GlcNAc

RayBio® Human Glycosylation Antibody Array L-4 List

number	name	number	name	number	name	number	name	number	name
1	Pos 1a	61	Laminin 2 alpha	121	MN1	181	Orosomucoid 2	241	Plakophilin 1
2	Pos 2a	62	Laminin b2	122	Moesin	182	ORP150	242	Plastin L
3	Pos 3a	63	Laminin gamma 1	123	MP1	183	OSBP1	243	PLC-gamma 1
4	Neg	64	LAMP	124	MPCA	184	OSCAR	244	Pleckstrin
5	HSPA1A	65	LAMP1	125	MPO	185	OSM R beta	245	Plectin
6	HTRA1	66	LAMP2	126	MRP 1	186	Osteoadherin(2)	246	Plexin B1
7	Human Agrin	67	LAP3	127	MSH6	187	Oxytocin-neurophysin 1/OXT	247	Plexin B2
8	IBP160	68	LASP1	128	mTOR	188	p16 ARC	248	PLOD1
9	IDH1	69	Latent TGF beta bp2	129	MUCDHL	189	P20Sb3	249	PLOD2
10	IDH3A	70	LCAT	130	Multimerin 2	190	p23	250	PLS3
11	IFRD1	71	LCMT2	131	MyBPC3	191	p39	251	Plxdc2
12	IGF2BP2	72	LDHA	132	MYH2	192	P4HB	252	PNP
13	IGFBP7	73	LDHB	133	MYH6	193	p73	253	POR
14	IGSF4B	74	LEDGF	134	MYH7	194	PA2G4	254	PPCS
15	Ihh	75	LEKTI/SPINK5	135	MYHC	195	PABP	255	PPOX
16	ILK	76	LILRA3	136	MYL12B	196	PACS1	256	PPP2R1B
17	Inhibin beta	77	LIMS1	137	MYL3	197	PARVB	257	PPP2R4
18	Integrin b1	78	LMAN2	138	MYO5A	198	PCBP1	258	PRCP
19	Integrin beta 6	79	LMW-PTP/ACP1	139	Myoferlin	199	PCBP2	259	PRDM13
20	Intergrin a6	80	LOK	140	Myosin 18B	200	PCCA	260	PRDX 1
21	IQGAP1	81	LOX	141	Myotrophin	201	PCDH7	261	PRELP
22	IQGAP2	82	LOXL1	142	NABC1	202	PCDX8	262	PREP
23	IRE1	83	LRP 4	143	NAGLU	203	PCK2	263	PRG2
24	IRS2	84	LTA4H	144	NAP1L1	204	PCMT1	264	Prion protein PrP /PRNP
25	ISOC2	85	LTBP4	145	NAPRT1	205	PCNA	265	Profilin 1
26	ITGB4BP	86	Lubricin	146	NASP	206	PCPE-1	266	Properdin
27	ITIH1	87	LUZP1	147	NCAM2	207	PCSK9	267	Prosaposin
28	ITIH2	88	LYPA1	148	Nebulin	208	PCYOX1	268	Prostaglandin D Synthase/PTGDS
29	ITIH3	89	Lysozyme	149	Nectin-1	209	PDE1B	269	Proteasome 20S a+b
30	ITIH4 a	90	MAGI2	150	Nectin-3	210	PDIA6	270	Proteasome 20S alpha
31	JAM-A	91	MAGP-2	151	NEDD8	211	PDLIM1	271	Proteasome 20S alpha 5
32	JARID2	92	MAN1	152	Neogenin	212	PDLIM5	272	Proteasome 20S b7
33	Karyopherin beta 1	93	MANF	153	Nesprin2	213	PDZD2	273	Proteasome 26S S5
34	Keratin 36	94	Mannosidase II	154	Neurabin 1	214	PEBP4	274	Proteasome beta 1
35	Keratin 38	95	MAP1A	155	Neural Cadherin	215	PEPD	275	Proteasome subunit alpha 6/PSMA6
36	KHSRP	96	MAPRE1	156	Neurofibromin	216	PER1	276	Proteasome subunit beta 2/PSB2
37	KIAA0319L	97	MARCKS	157	Neurogranin	217	perilipin 3	277	Proteasome subunit beta 4/PSB4
38	KIAA1468	98	MASP3	158	Neuropeptide B	218	Perilipin-1	278	Protein C
39	KIAA1967	99	MBD2	159	Neuropilin-1	219	Periostin	279	Protein Z
40	KIF5B	100	MBP	160	Neurotrimin	220	Peroxiredoxin 2	280	Prouroguanylin
41	Kilon	101	MCAM	161	NF-M	221	Peroxiredoxin 3	281	PRSS23
42	KLK-B1	102	Mcl-1	162	Nidogen-2	222	Peroxiredoxin 5	282	PRSS3
43	KMD4B	103	MCM	163	NIT2	223	PF4V1	283	PRTN3
44	KMT2B	104	MCM5	164	NME3	224	PGAM1	284	PSMA1
45	KRT31	105	MCMP2	165	nNOS	225	PGAM2	285	PSMA2
46	KRT72	106	MDH1	166	Noelin	226	PGD	286	Pos 1b
47	Krt73	107	MDH2	167	Non-muscle Actin/Actin	227	PGDF/PHGDH	287	Pos 2b
48	KRT82	108	ME1	168	non-muscle Myosin IIA/Myosin	228	PGK-1	288	Pos 3b
49	KRT85 - N-terminal	109	MEP1A	169	Notch-2	229	PGLS-C-t	289	Neg
50	KRTDAP	110	Metallothionein	170	Notch-2 ICD	230	PGM1	290	PSMA4
51	KRTHA3B	111	Metavinculin	171	NPAS3	231	PGRPL	291	PSMA7
52	KSR1	112	MFAP4	172	NPM1	232	PHAP1	292	PSMB5
53	LAD	113	MFI2	173	NQO2	233	PSAT1	293	PSMC3
54	LAF4	114	mGLUR5	174	NT5C3	234	PI 3-Kinase C2 beta	294	PSMD1
55	LAIR1	115	MGP	175	Nucleobindin 1/NUCB1	235	pIgR	295	PSMD9
56	LAM b1	116	Mimecan	176	NUP98	236	PIK3IP1	296	PTEN
57	LAMA	117	MINPP1	177	OBCAM	237	PIN	297	PTK 7
58	Lamin A + C	118	MLCK	178	OIT3	238	PISD	298	PTMA
59	Lamin B1	119	MMR	179	Olfactomedin-2	239	PKLR	299	PTP gamma
60	Lamin B2	120	MMRN1	180	OTC	240	PLA2G1B	300	PTP kappa

RayBio® Human Glycosylation Antibody Array L-4 List ...continued

number	name	number	name	number	name	number	name
301	PTP mu	361	SDNSF	421	SUMO3	481	URB
302	PTPRS	362	SDPR	422	Symplekin	482	URB2
303	PTPRZ	363	Secretogranin V/SCG5	423	SynCAM	483	UROC1
304	PYGL	364	Semaphorin 6B	424	Syntaxin 7	484	UROD
305	PZP	365	Semaphorin 7A	425	TAB182	485	URP2
306	QDPR	366	Semenogelin I/SEMG1	426	TAGLN2	486	USP14
307	QPRT	367	Semenogelin II/SEMG2	427	Talin1	487	USP5
308	Quiescin Q6	368	Serpin A11	428	Talin1&2	488	Uteroglobin(1)
309	Rab7a	369	Serpin A7	429	TAX1BP3	489	Utrophin
310	Ran	370	Serpin B3/SCCA1	430	TBCA	490	VAP-1
311	RanGAP1	371	Serpin B6	431	TCEB2	491	VAP-A
312	RAP1AB	372	Serpin B8	432	Tcf20	492	VCP
313	Rbm15	373	Serpin F2	433	TCN1	493	VDAC1 / Porin
314	RCL	374	Serpin A10/ZPI	434	TCP1 eta	494	Versican isoform V0
315	Reg1A	375	SERPINB1	435	Tenascin C	495	Vimentin B
316	Reg3A	376	SerpinB4	436	Tenascin X(1)	496	VNN1
317	RHOC	377	SerpinE2	437	TFF2(1)	497	VSIG4
318	RhoGDI	378	SerRS	438	TGM3	498	WDR1
319	Ribonuclease A	379	SET	439	Thioredoxin-1	499	WISP2
320	Ribonuclease Inhibitor	380	SEZ6L2	440	THOP1	500	WNK2
321	Ribonuclease T2	381	SF20	441	Thymosin b10	501	YB1
322	RKIP	382	SH3BGRL	442	Titin	502	YY1
323	RNA Polymerase II/POLR2A	383	SH3BGRL3	443	TLS/FUS	503	ZBTB4
324	RNASE4	384	SHANK1	444	TMEM223	504	ZC3H4-N-t
325	RNASE6	385	SHC1	445	TOB2	505	ZC3H8
326	RPL10	386	SHIP	446	TOP2B	506	ZDHHC18
327	RPL10A	387	SHMT1	447	TPM4	507	ZNF671
328	RPL11	388	SHP-1	448	TPP1	508	Zyxin
329	RPL12	389	Siglec-1	449	Transaldolase 1/TALDO1	509	Neg
330	RPL14	390	SIGLEC14	450	Transketolase/TALDO	510	Neg
331	RPL17	391	SIM2	451	Transthyretin	511	Neg
332	RPL22	392	SIRP beta 1/CD172b	452	TRAP1	512	Neg
333	RPL5	393	Six3	453	TRAP220	513	Neg
334	RPL7A	394	SLC38A10	454	TRF 2	514	Neg
335	RPLP0	395	SLITRK1	455	Triosephosphate isomerase/TPIS	515	Neg
336	RPS10	396	SLURP1	456	Tropomyosin 3	516	Neg
337	RPS11	397	SMA	457	TRP-1	517	Neg
338	RPS12	398	SMC4	458	TRPS1	518	Neg
339	RPS19	399	SMPD4	459	Trypsinogeb-2	519	Neg
340	RPS2	400	SOD1	460	TrypsinPan	520	Neg
341	RPS20	401	SOD2	461	Tryptophanyl	521	Neg
342	RPS23	402	SOD-3	462	TSR2	522	Neg
343	RPS25	403	SOD4	463	TUBA6	523	Pos 1c
344	RPS28	404	Somatostatin	464	TWF2	524	Pos 2c
345	RPS3	405	SORD	465	TXNDC4	525	Pos 3c
346	RPS5	406	SorLA	466	TXNDC5	526	
347	RREB1	407	SOX4	467	TXNRD2	527	
348	RSU1	408	SP-D	468	UBE2D3	528	
349	S100A1	409	Spectrin beta-5	469	Ube2L3	529	
350	S100A11	410	SPEN	470	UBE2N/Ubc13	530	
351	S100A7	411	SPINK7	471	Ubiquitin	531	
352	S100A9	412	SPTBN1	472	UCH-L1	532	
353	S100P	413	Src(1)	473	UFM 1	533	
354	SAA	414	SREC-II	474	UGGT	534	
355	SAA4a	415	STAT3	475	UNC-13 Homolog D	535	
356	Salivary alpha amylase/aAmylase	416	Stathmin 1	476	UNC45A	536	
357	SAMSN1	417	Sterol carrier protein 2/SCP2	477	UNC5H4	537	
358	SBP-1	418	STI1	478	UPB1	538	
359	SBSN	419	STOM	479	UQCRB	539	
360	SDF4	420	SUCLG1	480	UQCRH	540	

VI. Interpretation of Results:

A. Explanation of Controls Spots

1) **Positive Control spots (POS1, POS2, POS3)** are standardized amounts of biotinylated IgGs printed directly onto the array. All other variables being equal, the Positive Control intensities will be the same for each sub-array. This allows for normalization based upon the relative fluorescence signal responses to a known control, much as "housekeeping" genes or proteins are used to normalize results in PCR or Western blots, respectively.

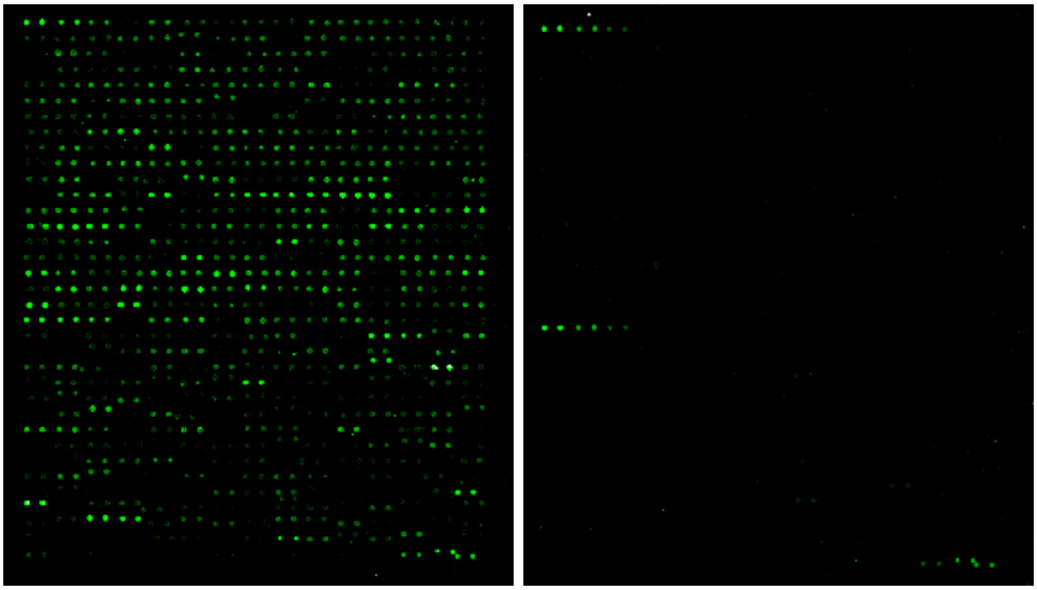
2) **Negative Control (NEG) spots** contain a protein-containing buffer (used to dilute antibodies printed on the array). Their signal intensities represent non-specific binding of the Biotin-conjugated lectin mixture and/or the Cy3-Conjugated Streptavidin. Negative control signal intensities are usually very close to background signals in each sub-array.

B. Typical Results

The following figure shows the RayBio® Human Glycosylation Antibody Array L-4 probed with a conditioned media sample. The images were captured using an Axon GenePix laser scanner. The strong signals in row 20 and the upper left and lower right corners of each array are Positive Controls, which can be used to identify the orientation and help normalize the results between arrays.

RayBio® Human Glycosylation Antibody Array L-4

Conditioned medium No Sample



RayBio Human Glycosylation Antibody Array L-4. Probed with biotinylated lectins: (b- Con A; DBA, PNA, UEA I, WGA)

If scanned using optimal settings, 3 distinct signal intensities will be seen: POS1>POS2>POS3. If all of these signals are of similar intensity, try increasing or decreasing laser power and/or signal gain settings.

C. Background Subtraction:

Once you have obtained the fluorescence intensity data, you should subtract the background and normalize to the Positive Control signals before proceeding to analysis.

Most laser fluorescence scanner software has an option to automatically measure the local background around each spot. For best results, we recommend comparing signal intensities representing the MEDIAN

background signals minus local background. If your resulting fluorescence signal intensity reports do not include these values (e.g., a column labeled as “MED532-B532”), you may need to subtract the background manually or change the default settings on your scanner’s data report menu.

D. Normalization of Array Data:

To normalize signal intensity data, one sub-array is defined as "reference" to which the other arrays are normalized. This choice is arbitrary. For example, in our Analysis Tool Software (described below), the array represented by data entered in the left-most column each worksheet is the default “reference array.”

You can calculate the normalized values as follows:

X(Ny) = X(y) * P1/P(y)

Where:
P1 = mean signal intensity of POS spots on reference array
P(y) = mean signal intensity of POS spots on Array "y"
X(y) = mean signal intensity for spot "X" on Array "y"
X(Ny) = normalized signal intensity for spot "X" on Array "y"

The RayBio® Analysis Tool software is available for use with data obtained using RayBio® Arrays. You can copy and paste your signal intensity data (with and without background) into the Analysis Tool, and it will automatically normalize signal intensities to the Positive Controls.

To order the Analysis Tool, please contact us toll-free at +1-888-494-8555 or info@raybiotech.com for more information.

E. Threshold of significant difference in expression:

After subtracting background signals and normalization to Positive Controls, comparison of signal intensities between and among array images can be used to determine relative differences in expression levels of each protein between samples or groups.

Any ≥1.5-fold increase or ≤0.65-fold decrease in signal intensity for a single analyte between samples or groups may be considered a measurable and significant difference in expression, provided that both sets of signals are well above background (Mean background + 2 standard deviations, accuracy ≈ 95%).

VII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak signal	Inadequate detection	Check laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettors and ensure correct preparation
	Short incubation times	Ensure sufficient incubation time and change sample incubation step to overnight
	Too low protein concentration in sample	Decrease dilution Or concentrate sample
	Improper storage of kit	Store kit at suggested temperature
High background	Sample is too concentrated	Use more diluted sample
	Excess of streptavidin	Make sure to use the correct amount of streptavidin
	Inadequate detection	Check laser power and PMT parameters
	Inadequate wash	Increase the volume of wash buffer and incubation time
Uneven signal	Bubbles formed during incubation	Avoid bubble formation during incubation
	Arrays are not completely covered by reagent	Completely cover arrays with solution

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