RayBio® Human Glycosylation Antibody Array 507

For the simultaneous detection of the glycosylation protein profile of 507 human proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

User Manual (Revised Dec 9, 2019)

Human Glycosylation Array 507
Cat# GAH-GCM-507-4 (4 Sample Kit)
Cat# GAH-GCM-507-8 (8 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

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 507, researchers can now efficiently obtain glycosylation profiles in their samples. This assay allows the simultaneous detection of glycosylation profiles of 507 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 507 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-507-4)	8 Sample Kit (GAH-GCM-507-8)
A	Human Glycosylation Array 507 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

** 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.

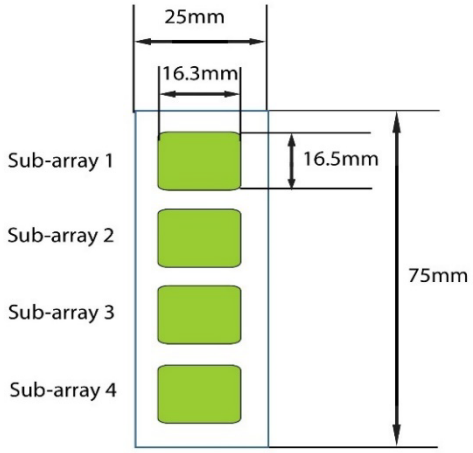
4. Transfer supernatant to a clean tube and store at -80°C.

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 507 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.

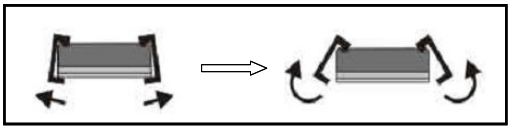
Note: Avoid exposure to light in Steps 13-20 by covering the Glass Slide Assembly with aluminum foil or incubate in dark room.

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

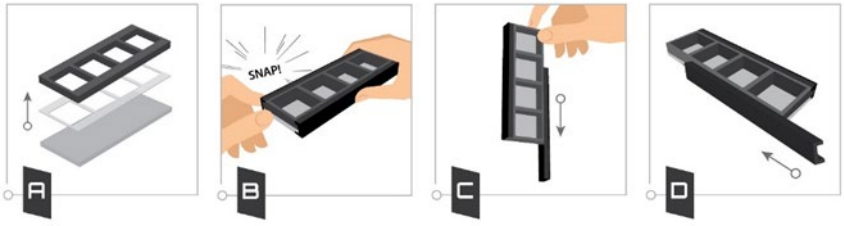
19. Allow glass chip to dry in a laminar flow hood protected from light for 20 minutes or until slide is completely dry. Place chip under an aluminum foil tent to protect it from light. Make sure the slides are absolutely dry before scanning or storage.

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 507 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
3	31	31	32	32	33	33	34	34	35	35	36	36	37	37	38	38	39	39	40	40	41	41	42	42	43	43	44	44	45	45
4	46	46	47	47	48	48	49	49	50	50	51	51	52	52	53	53	54	54	55	55	56	56	57	57	58	58	59	59	60	60
5	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	74	75	75
6	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84	85	85	86	86	87	87	88	88	89	89	90	90
7	91	91	92	92	93	93	94	94	95	95	96	96	97	97	98	98	99	99	100	100	101	101	102	102	103	103	104	104	105	105
8	106	106	107	107	108	108	109	109	110	110	111	111	112	112	113	113	114	114	115	115	116	116	117	117	118	118	119	119	120	120
9	121	121	122	122	123	123	124	124	125	125	126	126	127	127	128	128	129	129	130	130	131	131	132	132	133	133	134	134	135	135
10	136	136	137	137	138	138	139	139	140	140	141	141	142	142	143	143	144	144	145	145	146	146	147	147	148	148	149	149	150	150
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20	P-1b	P-1b	P-2b	P-2b	P-3b	P-3b	Neg	Neg	290	290	291	291	292	292	293	293	294	294	295	295	296	296	297	297	298	298	299	299	300	300
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34	496	496	497	497	498	498	499	499	500	500	501	501	502	502	503	503	504	504	505	505	506	506	507	507	508	508	509	509	510	510
35	511	511	512	512	513	513	514	514	515	515	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	P-3c	P-3c	P-2c	P-2c	P-1c	P-1c

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 507 List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	Positive 1a	61	CCR7	121	Eotaxin-2 / MPIF-2	181	GFR alpha-2	241	IL-1 R6 / IL-1 Rrp2
2	Positive 2a	62	CCR8	122	Eotaxin-3 / CCL26	182	GFR alpha-3	242	IL-1 R8
3	Positive 3a	63	CCR9	123	Epiregulin	183	GFR alpha-4	243	IL-1 R9
4	neg	64	CD14	124	ErbB2	184	GITR / TNFRF18	244	IL-1 ra
5	6Ckine	65	CD27 / TNFRSF7	125	ErbB3	185	GITR Ligand / TNFSF18	245	IL-1 sRI
6	Activin A	66	CD30 / TNFRSF8	126	ErbB4	186	Glucagon	246	IL-1 sRII
7	Activin B	67	CD30 Ligand / TNFSF8	127	Erythropoietin	187	Glut1	247	IL-2
8	Activin C	68	CD40 / TNFRSF5	128	E-Selectin	188	Glut2	248	IL-2 R alpha
9	Activin RIA / ALK-2	69	CD40 Ligand / TNFSF5 /CD154	129	FADD	189	Glut3	249	IL-2 R beta /CD122
10	Activin RIB / ALK-4	70	CD 163	130	FAM3B	190	Glut5	250	IL-2 R gamma
11	Activin RII A/B	71	Cerberus 1	131	Fas / TNFRSF6	191	Glypican 3	251	IL-3
12	Activin RIIA	72	Chem R23	132	Fas Ligand	192	Glypican 5	252	IL-3 R alpha
13	Adiponectin / Acrp30	73	Chordin-Like 1	133	FGF Basic	193	GM-CSF	253	IL-4
14	AgRP	74	Chordin-Like 2	134	FGF-BP	194	GM-CSF R alpha	254	IL-4 R
15	ALCAM	75	CLC	135	FGF R3	195	Granzyme A	255	IL-5
16	Angiogenin	76	CNTF	136	FGF R4	196	GREMLIN	256	IL-5 R alpha
17	Angiopoietin-1	77	CNTF R alpha	137	FGF R5	197	GRO	257	IL-6
18	Angiopoietin-2	78	Coagulation Factor III / Tissue Factor	138	FGF-4	198	GRO-a	258	IL-6 R
19	Angiopoietin-4	79	CRIM 1	139	FGF-5	199	Growth Hormone (GH)	259	IL-7
20	Angiopoietin-like 1	80	Cripto-1	140	FGF-6	200	Growth Hormone R (GHR)	260	IL-7 R alpha
21	Angiopoietin-like 2	81	CRTH-2	141	FGF-7 / KGF	201	HB-EGF	261	IL-8
22	Angiopoietin-like Factor	82	Cryptic	142	FGF-8	202	HCC-4 / CCL16	262	IL-9
23	Angiostatin	83	Csk	143	FGF-9	203	HCR / CRAM-A/B	263	IL-10
24	APJ	84	CTACK / CCL27	144	FGF-10 / KGF-2	204	Hepassocin	264	IL-10 R alpha
25	APRIL	85	CTGF / CCN2	145	FGF-11	205	GLO-1	265	IL-10 R beta
26	AR (Amphiregulin)	86	CTLA-4 /CD152	146	FGF-12	206	HGF	266	IL-11
27	Artemin	87	CV-2 / Crossveinless-2	147	FGF-13 1B	207	HGFR	267	IL-12 p40
28	Axl	88	CXCL14 / BRAK	148	FGF-16	208	HRG-alpha	268	IL-12 p70
29	B7-1 /CD80	89	CXCL16	149	FGF-17	209	HRG-beta 1	269	IL-12 R beta 1
30	BAFF R / TNFRSF13C	90	CXCR1 / IL-8 RA	150	FGF-18	210	HVEM / TNFRSF14	270	IL-12 R beta 2
31	BCMA / TNFRSF17	91	CXCR2 / IL-8 RB	151	FGF-19	211	I-309	271	IL-13
32	BD-1	92	CXCR3	152	FGF-20	212	ICAM-1	272	IL-13 R alpha 1
33	BDNF	93	CXCR4 (fusin)	153	FGF-21	213	ICAM-2	273	IL-13 R alpha 2
34	beta-Catenin	94	CXCR5 /BLR-1	154	FGF-23	214	ICAM-3 (CD50)	274	IL-15
35	BAX	95	CXCR6	155	FLRG	215	ICAM-5	275	IL-15 R alpha
36	beta-NGF	96	D6	156	Flt-3 Ligand	216	IFN-alpha / beta R1	276	IL-16
37	BIK	97	DAN	157	Follistatin	217	IFN-alpha / beta R2	277	IL-17
38	BLC / BCA-1 / CXCL13	98	DANCE	158	Follistatin-like 1	218	IFN-beta	278	IL-17B
39	BMP-2	99	DcR3 / TNFRSF6B	159	Fractalkine	219	IFN-gamma	279	IL-17B R
40	BMP-3	100	Decorin	160	Frizzled-1	220	IFN-gamma R1	280	IL-17C
41	BMP-3b / GDF-10	101	Dkk-1	161	Frizzled-3	221	IGFBP-1	281	IL-17D
42	BMP-4	102	Dkk-3	162	Frizzled-4	222	IGFBP-2	282	IL-17E
43	BMP-5	103	Dkk-4	163	Frizzled-5	223	IGFBP-3	283	IL-17F
44	BMP-6	104	DR3 / TNFRSF25	164	Frizzled-6	224	IGFBP-4	284	IL-17R
45	BMP-7	105	DR6 / TNFRSF21	165	Frizzled-7	225	IGFBP-6	285	IL-17RC
46	BMP-8	106	Dtk	166	Galectin-3	226	IGFBP-rp1 / IGFBP-7	286	Positive 1b
47	BMP-15	107	EDA-A2	167	GASP-1 / WFIKKNRP	227	IGF-I	287	Positive 2b
48	BMPR-IA / ALK-3	108	EDAR	168	GASP-2 / WFIKKN	228	IGF-I SR	288	Positive 3b
49	BMPR-IB / ALK-6	109	EDG-1	169	GCP-2 / CXCL6	229	IGF-II	289	neg
50	BMPR-II	110	EGF	170	GCSF	230	IGF-II R	290	IL-17RD
51	BTC	111	EGF R / ErbB1	171	G-CSF R / CD 114	231	IL-1 alpha	291	IL-18 BPa
52	Cardiotrophin-1 / CT-1	112	EG-VEGF / PK1	172	GDF1	232	IL-1 beta	292	IL-18 R alpha /IL-1 R5
53	CCL14 / HCC-1 / HCC-3	113	EMAP-II	173	GDF3	233	IL-1 F5 / FIL1delta	293	IL-18 R beta /AcPL
54	CCL28 / VIC	114	ENA-78	174	GDF5	234	IL-1 F6 / FIL1 epsilon	294	IL-19
55	CCR1	115	Endocan	175	GDF8	235	IL-1 F7 / FIL1 zeta	295	IL-20
56	CCR2	116	Endoglin / CD105	176	GDF9	236	IL-1 F8 / FIL1 eta	296	IL-20 R alpha
57	CCR3	117	Endostatin	177	GDF11	237	IL-1 F9 / IL-1 H1	297	IL-20 R beta
58	CCR4	118	Endothelin	178	GDF-15	238	IL-1 F10 / IL-1HY2	298	IL-21
59	CCR5	119	EN-RAGE	179	GDNF	239	IL-1 R3 / IL-1 R AcP	299	IL-21 R
60	CCR6	120	Eotaxin / CCL11	180	GFR alpha-1	240	IL-1 R4 / ST2	300	IL-22

RayBio® Human Glycosylation Antibody Array 507 List ...continued

Number	Name	Number	Name	Number	Name	Number	Name
301	IL-22 BP	361	MMP-2	421	RANK / TNFRSF11A	481	TMEFF1 / Tomoregulin-1
302	IL-22 R	362	MMP-3	422	RANTES	482	TMEFF2
303	IL-23	363	MMP-7	423	RELM beta	483	TNF-alpha
304	IL-23 R	364	MMP-8	424	RELT / TNFRSF19L	484	TNF-beta
305	IL-24	365	MMP-9	425	ROBO4	485	TNF RI / TNFRSF1A
306	IL-26	366	MMP-10	426	S100 A8/A9	486	TNF RII / TNFRSF1B
307	IL-27	367	MMP-11 /Stromelysin-3	427	S100A10	487	TRADD
308	IL-28A	368	MMP-12	428	SAA	488	TRAIL / TNFSF10
309	IL-29	369	MMP-13	429	SCF	489	TRAIL R1 / DR4 / TNFRSF10A
310	IL-31	370	MMP-14	430	SCF R /CD117	490	TRAIL R2 / DR5 / TNFRSF10B
311	IL-31 RA	371	MMP-15	431	SDF-1 / CXCL12	491	TRAIL R3 / TNFRSF10C
312	BACE-1	372	MMP-16 / MT3-MMP	432	sFRP-1	492	TRAIL R4 / TNFRSF10D
313	FACX	373	MMP-19	433	sFRP-3	493	TRANCE
314	Insulin	374	MMP-20	434	sFRP-4	494	TREM-1
315	Insulin R	375	MMP-24 / MT5-MMP	435	sgp130	495	TROY / TNFRSF19
316	Insulysin / IDE	376	MMP-25 / MT6-MMP	436	SIGIRR	496	TSG-6
317	IP-10	377	MSP alpha Chain	437	Siglec-5/CD170	497	TSLP R
318	I-TAC / CXCL11	378	Musk	438	Siglec-9	498	TWEAK / TNFSF12
319	Kininostatin / kininogen	379	NAP-2	439	SLPI	499	TWEAK R / TNFRSF12
320	Kremen-1	380	NCAM-1 / CD56	440	Smad 1	500	Ubiquitin+1
321	Kremen-2	381	Neuritin	441	Smad 4	501	uPA
322	Latent TGF-beta bp1	382	NeuroD1	442	Smad 5	502	uPAR
323	LBP	383	Neuropilin-2	443	Smad 7	503	Vasorin
324	Lck	384	Neurturin	444	Smad 8	504	VCAM-1 (CD106)
325	LECT2	385	NGF R	445	Prdx6	505	VE-Cadherin
326	Lefty - A	386	Nidgen-1	446	Soggy-1	506	VEGF
327	Leptin (OB)	387	NOV / CCN3	447	Sonic Hedgehog (Shh N-terminal)	507	VEGF R2 (KDR)
328	Leptin R	388	NrCAM	448	SPARC	508	VEGF R3
329	LFA-1 alpha	389	NRG1 Isoform GGF2	449	Spinesin	509	VEGF-B
330	LIF	390	NRG2	450	TACI / TNFRSF13B	510	VEGF-C
331	LIF R alpha	391	NRG3	451	Tarc	511	VEGF-D
332	LIGHT / TNFSF14	392	NT-3	452	TCCR / WSX-1	512	VEGI / TNFSF15
333	Lipocalin-1	393	NT-4	453	TECK / CCL25	513	WIF-1
334	Lipocalin-2	394	Orexin A	454	TFPI	514	WISP-1 / CCN4
335	LRP-1	395	Orexin B	455	TGF-alpha	515	XEDAR
336	LRP-6	396	OSM	456	TGF-beta 1	516	Neg
337	L-Selectin (CD62L)	397	Osteoactivin / GPNMB	457	TGF-beta 2	517	Neg
338	Lymphotactin / XCL1	398	Osteocrin	458	TGF-beta 3	518	Neg
339	Lymphotoxin beta / TNFSF3	399	Osteoprotegerin / TNFRSF11B	459	TGF-beta 5	519	Neg
340	Lymphotoxin beta R / TNFRSF3	400	OX40 Ligand / TNFSF4	460	TGF-beta RI / ALK-5	520	Neg
341	MAC-1	401	PARC / CCL18	461	TGF-beta RII	521	Neg
342	MCP-1	402	PD-ECGF	462	Grb2	522	Neg
343	MCP-2	403	PDGF R alpha	463	TGF-beta RIII	523	P-3c
344	MCP-3	404	PDGF R beta	464	Thrombopoietin (TPO)	524	P-2c
345	MCP-4 / CCL13	405	PDGF-AA	465	TPX	525	P-1c
346	M-CSF	406	PDGF-AB	466	Thrombospondin-1		
347	M-CSF R	407	PDGF-BB	467	Thrombospondin-2		
348	MDC	408	PDGF-C	468	Thrombospondin-4		
349	MFG-E8	409	PDGF-D	469	Thymopoietin		
350	MFRP	410	PECAM-1 /CD31	470	Tie-1		
351	MICA	411	Pentraxin3 / TSG-14	471	Tie-2		
352	MIF	412	Persephin	472	TIMP-1		
353	MIG	413	PF4 / CXCL4	473	TIMP-2		
354	MIP-1a	414	PlGF	474	TIMP-3		
355	MIP-1b	415	PLUNC	475	TIMP-4		
356	MIP-1d	416	Pref-1	476	TL1A / TNFSF15		
357	MIP 2	417	Progranulin	477	TLR1		
358	MIP-3 alpha	418	Prolactin	478	TLR2		
359	MIP-3 beta	419	P-selectin	479	TLR3		
360	MMP-1	420	RAGE	480	TLR4		

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.

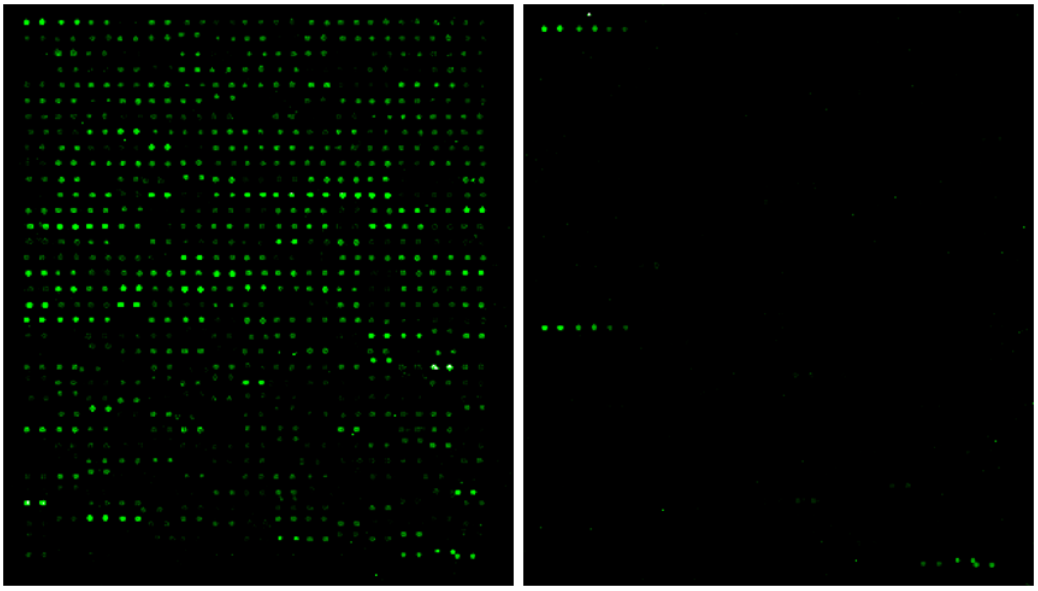
2) **Negative Control (NEG) spots** contain a protein-containing buffer (used to dilute antibodies printed on the array). Their signal intensities represent non-specific binding of the Biotin-conjugated lectin mixture and/or the Cy3-Conjugated Streptavidin. Negative control signal intensities are usually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

B. Typical Results

The following figure shows the RayBio® Human Glycosylation Antibody Array 507 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 507

Conditioned medium No Sample



RayBio Human Glycosylation Antibody Array 507. 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	Avo id bubble formation during incubation
	Arrays are not completely covered by reagent	Completely cover arrays with solution

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