RayBio® Human Protein S-Acylation Antibody Array 1

For Simultaneously Detecting the Relative Levels of S-Acylation (or S-Palmitoylation) of 507 Human Proteins

User Manual
Revised Mar. 22nd, 2024

Cat#: AAH-ACYL-G1-4 (4 Sample Kit)
Cat#: AAH-ACYL-G1-8 (8 Sample Kit)

Please read manual carefully before starting experiment

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RayBio® Human Protein S-Acylation Antibody Array 1 Protocol

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I. Introduction

Protein S-acylation (or protein S-palmitoylation) plays an unusually prominent role in cell signaling, development and growth. RayBio® Human Protein S-Acylation Antibody Array 1 is specifically designed for simultaneous identification of the relative levels of S-Acylation of cysteine of 507 different human proteins in cell lysate, culture supernatant, serum, plasma and other biological samples. By monitoring the changes in protein S-Acylation in your experimental model system, you can verify pathway activation in your cell lines without spending excess time and effort performing an analysis of immunoprecipitation and/or Western Blot.

The array kit uses a modified 'biotin-switch' (acyl-biotinyl exchange (ABE)) method (Jaffrey *et al*.) to allow for the direct visualization of S-acylated proteins on the antibody array. Prior to running the array, the samples are labeled. Unmodified free cysteines on proteins in the sample are first blocked. S-acylated cysteines are then selectively reduced for specific labeling with biotin-maleimide reagents, which irreversibly bind to the cysteine thiol that was S-acylated. Biotinylation of the newly formed thiol groups can then be detected on the antibody array. If desired, avidin resin can be used to selectively enrich S-acylated proteins/peptides labeled with biotin. The biotin labeled sample is added into antibody array glass slide wells. The antibody array slide wells are washed. After incubation with a fluorescent dye-conjugated streptavidin (Cy3 equivalent), the slides can then be imaged using a laser scanner, such as the Axon GenePix, using the Cy3 channel.

II. Materials Provided

Store kit at ≤ -20 °C immediately upon arrival. Kit must be used within the 6 months expiration date.

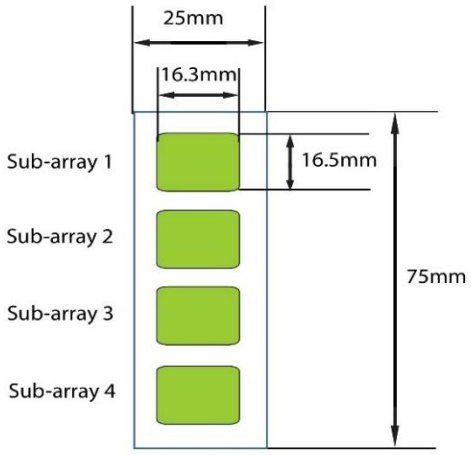
ITEM	COMPONENT	AAH-ACYL-G1-4	AAH-ACYL-G1-8	STORAGE TEMPERATURE AFTER THAWING**
1	RayBio Glass Slide*	1	2	≤ -20 °C
2	Blocking Buffer	1 bottle (8 ml)	2 bottles (8 ml)	
4	Fluorescent Dye-Conjugated Streptavidin (Cy3 equivalent)	1 vial	2 vials	2-8℃
5	20X Wash Buffer I Concentrate	1 bottle (30 ml)		2-8℃
6	20X Wash Buffer II Concentrate	1 bottle (30 ml)		
7	Wash Buffer III	1 bottle (30 ml)		
8	2X Cell Lysis Buffer Concentrate	1 bottle (10ml)		2-8℃
9	Protease Inhibitor Cocktail	1 vial		≤ -20 °C
10	S-Acylation Buffer A	12 ml		RT
11	S-Acylation Buffer B	3 ml		RT
12	S-Acylation Buffer C	25 ml		RT
13	S-Acylation Buffer D	12 ml		RT
14	S-Acylation Buffer E	12 ml		RT
15	S-Acylation Blocking Reagent	2 vials, enough for 2 separate experiments. Crystalline solid.		Prepare immediately prior to use. Do not store.
16	S-Acylation Reduction Reagent	2 bottles, enough for 2 separate experiments. Crystalline solid.		Prepare immediately prior to use. Do not store.
17	S-Acylation Labeling Reagent	2 vials, enough for 2 separate experiments. Crystalline solid.		Prepare immediately prior to use. Do not store.
Other Kit Components: Adhesive film				

*Each slide contains 4 identical subarrays
**For up to 3 months (unless stated otherwise) or until expiration date

III. Additional Materials Required

- Acetone, ≥ 98% (hazardous)
- 1.5 mL microcentrifuge tubes
- 15 mL tubes (polypropylene)
- 10 mL graduated cylinders (X2)
- Benchtop centrifuge and microcentrifuge (4°C)
- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation
- Water bath or heat block
- Shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- Plastic box
- 50 ml centrifuge tube
- Isopropanol (2-propanol)

Layout of Array Glass Slide



4 printed sub-arrays per glass chip

IV. Reagent Preparation

1. **Protease Inhibitor Cocktail:** Briefly spin down the Protease Inhibitor Cocktail vial before use. Add 60 µl of 1X Cell Lysis Buffer to the vial to prepare a 100X Protease Inhibitor Cocktail Concentrate.
2. **2X Cell Lysis Buffer:** The 2X Cell Lysis Buffer should be diluted 2-fold with deionized or distilled water to prepare a 1X Cell Lysis Buffer solution. Then, add 20 µl of the Protease Inhibitor Cocktail Concentrate into 2 ml of the 1X Cell Lysis Buffer to prepare a 1X Cell Lysis Buffer with Protease Inhibitor Cocktail solution. Mix well before use.
3. **S-Acylation Blocking Reagent:** Make fresh. Spin briefly, add 50 µl Acylation Buffer B, vortex until all crystals are dissolved completely, then transfer everything into 5 ml S-Acylation Buffer A, mix well.
4. **S-Acylation Reduction Reagent:** Make fresh. Spin briefly, add 10mL S-Acylation Buffer C, vortex until all crystals are dissolved completely, mix well.
5. **S-Acylation Labeling Reagent:** Make fresh. Spin briefly, add 100 µL dH_2O, vortex until all crystals are dissolved completely.
6. **Acetone (not included):** pre-chilled (-20°C).
7. **4:1 acetone/water mixture:** 4 parts acetone mixed with 1 part dH_2O, pre-chilled (-20°C).
8. **20X Wash Buffer I or II:** If the 20X Wash Buffer Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 25 ml of the 20X Wash Buffer Concentrate into deionized or distilled water to yield 500 ml of 1X Wash Buffer.
9. **Fluorescent dye-Conjugated Streptavidin (Cy3 equivalent):** Briefly spin down the fluorescent dye-Conjugated Streptavidin vial before use. Add 180 µl of Blocking Buffer to the vial to prepare a Streptavidin Concentrate. Pipette up and down to mix gently. Transfer all Streptavidin Concentrate to a tube with 1.7 ml of Blocking Buffer to prepare a 1X Fluorescent dye-Conjugated Streptavidin solution. Mix gently.

V. Overview and General Considerations

A. <u>Preparation of Samples</u>

1. Cell lysate preparation: Cells can be prepared using the following convention.

For attached cells, remove the supernatant from the cell culture, and wash the cells twice with cold 1X PBS (for cells in suspension, pellet the cells by spinning down at 1500 rpm for 10 min). Make sure to remove any remaining PBS. Then, solubilize the cells at $2x10^7$ cells/ml in the 1X Cell Lysis Buffer with Protease Inhibitor Cocktail solution. Pipette up and down to resuspend the cells, and rock the lysates gently at 2–8 °C for 30 min. Transfer the lysates to microcentrifuge tubes and centrifuge at 14,000 x g for 5 min.

It is recommended that sample protein concentrations be determined using a total protein assay. Lysates should be used immediately or aliquoted and stored at –80 °C. Thawed lysates should be kept on ice prior to use.

2. Biotinylation of S-acylated cysteines.

This kit contains enough reagent to label 40 samples containing 100-200 µg of total protein each.

1. Prepare 100 µl sample with total protein concentration at 1-2 mg/mL. It is recommended to label samples with equivalent protein concentrations.
2. Add 200 µl prepared S-Acylation Blocking Buffer (use fresh reagent, prepared immediately prior to use) into each sample. Incubate the samples in dark at 50 °C on a shaker with gentle rocking for 30 minutes.
3. Precipitate protein by adding 1200 µl (4 volume) pre-chilled (-20°C) acetone for each sample. Mix thoroughly by inversion followed by incubation at -20°C for 1 hour.
4. Centrifuge at 14,000 × g for 10 minutes at 4°C.
5. Carefully dispose of the supernatant, without dislodging the protein pellet.
6. Add 500 µl pre-chilled 4:1 acetone/water mixture to wash the pellet. Repeat steps 4 and 5.
7. Repeat step 6 to wash the pellet one more time.
8. Allow the acetone to evaporate from the uncapped tube at room temperature for 30 minutes. Do not over-dry pellet, or it may not be dissolved properly.
9. Reconstitute the pellet in 40 µl S-Acylation Buffer D.
10. Add 160 µl S-Acylation Reducing Buffer (use fresh reagent, prepared immediately prior to use), Incubate for 1 hour at 37 °C. (Option: At this step samples can incubate with reducing buffer at room temperature overnight for optimal result)
11. Add 4 µl S-Acylation Labeling Buffer (use fresh reagent, prepared immediately prior to use) to the reconstituted sample and incubate for 2 hours at room temperature with gentle rotation.
12. Repeat steps 3-5. Allow the acetone to evaporate from the uncapped tube at room temperature for 30 minutes. Do not over-dry pellet, or it may not be dissolved properly.
13. Reconstitute each protein pellet in 40 µl S-Acylation Buffer E. Vortex the tube several times and then quickly spin down (it is normal to have undissolved protein pellet). Transfer supernatant to new tubes. The sample is now ready for analysis by antibody array. The labeled sample can be stored at -20°C for future analysis.
14. Dilute each sample 10 folds with the antibody array blocking buffer (Item 2).

If you experience high background, you may further dilute your sample.

B. <u>Handling Glass Slides</u>

- The microarray slides are very sensitive. Do not touch the array surface with tips, forceps or hands. Hold the slides by the edges only.
- Handle all buffers and slides with latex free gloves.
- Avoid breaking the glass slide.
- Maintain a clean environment.

C. <u>Incubation</u>

- Completely cover the array area with sample or buffer during incubation and cover the incubation chamber with the adhesive film or plastic sheet protector to avoid drying.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Cover the incubation chamber with the adhesive film during incubation, particularly when the incubation is more than 2 hours.
- Avoid cross-contamination from overflowing solution to neighboring wells.

- Incubation steps such as step 2 (sample incubation, page 10), or step 6 (Fluorescent dye-Conjugated Streptavidin incubation, page 11) may be done at 4 °C overnight. Please make sure to cover the incubation chamber tightly to prevent evaporation.
- Avoid exposing the array slide to light from step 6 in page 11 on.

VI. Protocol

A. Dry the Glass Slide

Open the box containing the Glass Slide with Frame and take it out. Then let it air dry for 1 hour in a clean environment before use.

Note: *Protect the slide from dust or other contaminants.*

B. Blocking and Incubation

1. Add 400 µl of 1X Blocking Buffer to each well and incubate at room temperature with gentle shaking for 30 min to block the slides. Make sure no bubbles are in the wells.

2. Decant the Blocking Buffer from each well (make sure to remove all of the buffer). Add 400 µl of each sample into appropriate wells. Incubate the arrays with sample at room temperature with gentle shaking for 2 hours or at 4 °C overnight.

   Note: We recommend Dilute each sample 10 folds with the antibody array blocking buffer (Item 2). ***Make sure there are no bubbles in the wells.***

   Note: The amount of sample used depends on the abundance of target proteins. More sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further. The optimal sample dilution must be determined empirically by the researcher.

   Note: Incubation may be done at 4 °C overnight.

3. Decant the samples from each well, and wash 3 times, 5 min per wash, with 800 µl of 1X Wash Buffer I at room temperature with gentle shaking.

   Note: Avoid the solution overflowing into neighboring wells.

4. Put the Glass Slide with Frame into a box with Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle shaking for 20 min.

5. Decant the Wash Buffer I from each well. Put the Glass Slide with Frame into a box with Wash Buffer II (cover the whole glass slide and frame with Wash Buffer II), and wash 2 times, 5 min per wash, at room temperature with gentle shaking.

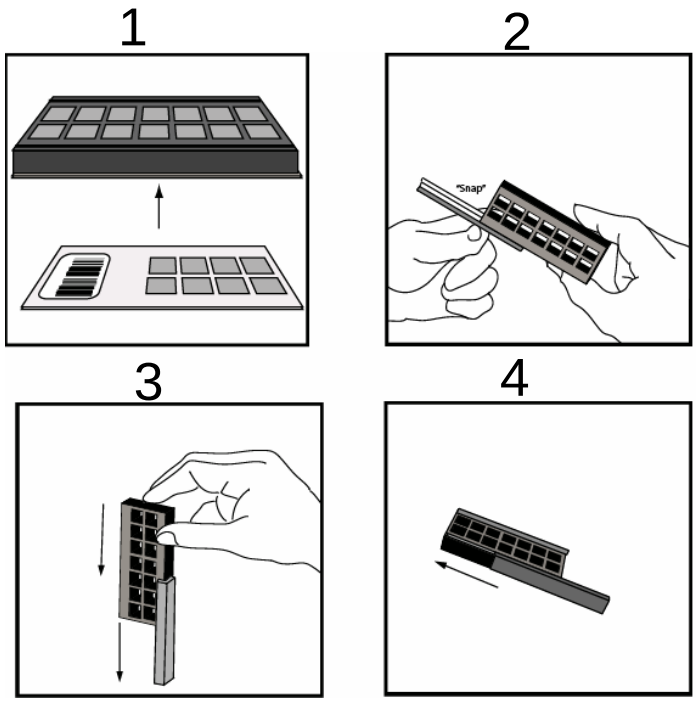
6. Remove all of Wash Buffer II from each well. Add 400 µl of the 1X Fluorescent dye-Conjugated Streptavidin solution to each subarray. Cover the incubation chamber with the Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in a dark room.

Note: Avoid exposing the array slide to light from this step forward.

7. Incubate at room temperature with gentle shaking for 2 hours in the dark.

Note: Incubation may be done at 4 °C overnight.

8. Decant the Fluorescent dye-Conjugated Streptavidin solution and disassemble the Glass Slide and Frame by removing the incubation frame and chamber from the slide as illustrated below.



Note: You may assemble and disassemble the glass slide into an incubation chamber and glass slide using the following steps.

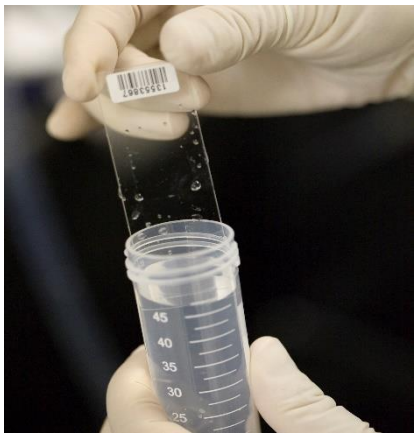
1. *To assemble, apply the incubation chamber to the slide with the printed side facing upward as illustrated in (1) above.*

2. *Gently snap one edge of a snap-on side as shown in (2).*

3. *Adjust the position of the snap-on by gently pressing the edge of the snap-on side against a lab bench and pushing down as shown in (3).*

4. *Repeat steps 2 – 3 with a second snap-on as shown in (4).*

9. Gently put the glass slide into a 50 ml centrifuge tube or a plastic box with 40 ml of 1X Wash Buffer I as illustrated below. Gently roll or shake the tube for 5 min. Remove the Wash Buffer I. Repeat 2 more times for a total of 3 washes.



10. Wash the glass slide with 40 ml of Wash Buffer II for 5 min. Repeat one more time for a total of 2 washes.

11. Finally, wash the glass slide with 40 ml of deionized or distilled water.

C. Fluorescence Detection

1. To dry the glass slide, do one of the following:
   a. Put the glass slide into a 50 ml centrifuge tube and centrifuge at 1,000 rpm for 3 min

      or

   b. Apply a compressed N_2 stream, or let glass slide air dry completely under clean air conditions (protected from light).

   Make sure the slides are absolutely dry before scanning.

2. Image the slides using a laser scanner, such as the Axon GenePix, using the Cy3 channel.

   Note: We recommend scanning the slides immediately after completing the experiment. Slides can also be stored at –20 °C in the dark for several days. If you do not have a laser scanner, we can scan and extract the data for free for you.

   Note: Put the glass slide into a tube with 40 ml of 30% Wash Buffer III in isopropanol (add 15 ml of Wash Buffer III to a tube with 35 ml of isopropanol and mix well) and incubate for 10 min at room temperature if the background is not even or too high

(cover the tube with aluminum foil to avoid exposure to light or incubate in a dark room). Dry the slide completely and re-scan the slide.

VII. Interpretation of Results

A biotinylated protein produces positive control signals, which can be used to identify the orientation of the slide and to normalize the results for comparison of different wells.

The antibody affinity to its target varies significantly between different antibodies. The fluorescence intensity detected on the array with each antibody depends on this affinity; therefore, the signal intensity comparison can only be performed within the same antibody/antigen system and not between different antibodies on the same slide.

RayBio Human Protein S-Acylation Antibody Array 1 Array 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	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11
2	12	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26
3	27	27	28	28	29	29	30	30	31	31	32	32	33	33	34	34	35	35	36	36	37	37	38	38	39	39	40	40	41	41
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5	57	57	58	58	59	59	60	60	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70	71	71
6	72	72	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84	85	85	86	86
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35	503	503	504	504	505	505	506	506	507	507	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	POS3	POS3	POS2	POS2	POS1	POS1

RayBio Human Protein S-Acylation Antibody Array 1 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	6Ckine	74	F3	147	FGF-19	220	IGFBP-4	293	IL-22 BP	366	MMP-20	439	Shh-N
2	Activin A	75	CRIM 1	148	FGF-20	221	IGFBP-6	294	IL-22 R	367	MMP-24	440	SPARC
3	Activin B	76	Cripto-1	149	FGF-21	222	IGFBP-rp1	295	IL-23	368	MMP-25	441	Spinesin
4	Activin C	77	CRTH-2	150	FGF-23	223	IGF-I	296	IL-23 R	369	MSPa	442	TACI
5	Activin RIA	78	Cryptic	151	FLRG	224	IGF-I R	297	IL-24	370	Musk	443	Tarc
6	Activin RIB	79	Csk	152	Flt-3 Ligand	225	IGF-II	298	IL-26	371	NAP-2	444	TCCR
7	EYA2	80	CTACK	153	Follistatin	226	IGF-II R	299	IL-27	372	NCAM-1	445	TECK
8	Activin RIIA	81	CTGF	154	Follistatin-like 1	227	IL-1 alpha	300	IL-28A	373	Neuritin	446	TFPI
9	Adiponectin	82	CTLA-4	155	Fractalkine	228	IL-1 beta	301	IL-29	374	NeuroD1	447	TGF-alpha
10	AgRP	83	CV-2	156	Frizzled-1	229	IL-1 F5	302	IL-31	375	Neuropilin-2	448	TGF-beta 1
11	ALCAM	84	CXCL14	157	Frizzled-3	230	IL-1 F6	303	IL-31 RA	376	Neurturin	449	TGF-beta 2
12	Angiogenin	85	CXCL16	158	Frizzled-4	231	IL-1 F7	304	BACE-1	377	NGF R	450	TGF-beta 3
13	Angiopoietin-1	86	CXCR1	159	Frizzled-5	232	IL-1 F8	305	FACX	378	Nidogen-1	451	ATP2B1
14	Angiopoietin-2	87	CXCR2	160	Frizzled-6	233	IL-1 F9	306	Insulin	379	NOV	452	TGF-beta RI
15	Angiopoietin-4	88	CXCR3	161	Frizzled-7	234	IL-1 F10	307	Insulin R	380	NrCam	453	TGF-beta RII
16	ANGPTL1	89	CXCR4	162	Galectin-3	235	IL-1 R3	308	Insulysin	381	GGF2	454	Grb2
17	ANGPTL2	90	CXCR5	163	GASP-1	236	IL-1 R4	309	IP-10	382	NRG2	455	TGF-beta RIII
18	ANGPTL7	91	CXCR6	164	GASP-2	237	IL-1 R6	310	I-TAC	383	NRG3	456	Thrombopoietin
19	Angiostatin	92	D6	165	GCP-2	238	IL-1 R8	311	Kininostatin	384	NT-3	457	Thyroid Peroxidase
20	APJ	93	DAN	166	GCSF	239	IL-1 R9	312	Kremen-1	385	NT-4	458	Thrombospondin-1
21	APRIL	94	DANCE	167	G-CSF R	240	IL-1 ra	313	Kremen-2	386	Orexin A	459	Thrombospondin-2
22	Amphiregulin	95	DcR3	168	GDF1	241	IL-1 RI	314	LTBP1	387	Orexin B	460	Thrombospondin-4
23	Artemin	96	Decorin	169	GDF3	242	IL-1 RII	315	LBP	388	OSM	461	Thymopoietin
24	Axl	97	Dkk-1	170	GDF5	243	IL-2	316	Lck	389	Osteoactivin	462	Tie-1
25	B7-1	98	Dkk-3	171	GDF8	244	IL-2 R alpha	317	LECT2	390	Osteocrin	463	Tie-2
26	BAFF R	99	Dkk-4	172	GDF9	245	IL-2 R beta	318	Lefty-A	391	Osteoprotegerin	464	TIMP-1
27	BCMA	100	DR3	173	GDF11	246	IL-2 R gamma	319	Leptin	392	OX40 Ligand	465	TIMP-2
28	BD-1	101	DR6	174	GDF-15	247	IL-3	320	Leptin R	393	PARC	466	TIMP-3
29	BDNF	102	Dtk	175	GDNF	248	IL-3 R alpha	321	LFA-1 alpha	394	PD-ECGF	467	TIMP-4
30	beta-Catenin	103	EDA-A2	176	GFR alpha-1	249	IL-4	322	LIF	395	PDGF R alpha	468	DEFA5
31	Bax	104	EDAR	177	GFR alpha-2	250	IL-4 R	323	LIF R alpha	396	PDGF R beta	469	TLR1
32	beta-NGF	105	EDG-1	178	GFR alpha-3	251	IL-5	324	LIGHT	397	PDGF-AA	470	TLR2
33	BIK	106	EGF	179	GFR alpha-4	252	IL-5 R alpha	325	Lipocalin-1	398	PDGF-AB	471	TLR3
34	BLC	107	EGF R	180	GITR	253	IL-6	326	Lipocalin-2	399	PDGF-BB	472	TLR4
35	BMP-2	108	EG-VEGF	181	GITR Ligand	254	IL-6 R	327	LRP-1	400	PDGF-C	473	TMEFF1
36	BMP-3	109	EMAP-II	182	CBR1	255	IL-7	328	LRP-6	401	PDGF-D	474	TMEFF2
37	BMP-3b	110	ENA-78	183	Glut1	256	IL-7 R alpha	329	L-Selectin	402	PECAM-1	475	TNF-alpha
38	BMP-4	111	Endocan	184	Glut2	257	IL-8	330	Lymphotactin	403	Pentraxin3	476	TNF-beta
39	BMP-5	112	Endoglin	185	Glut3	258	IL-9	331	LTB	404	Persephin	477	TNF RI
40	BMP-6	113	Endostatin	186	Glut5	259	IL-10	332	LTBR	405	PF4	478	TNF RII
41	BMP-7	114	Endothelin	187	Glypican 3	260	IL-10 R alpha	333	MAC-1	406	PlGF	479	TRADD
42	BMP-8	115	EN-RAGE	188	Glypican 5	261	IL-10 R beta	334	MCP-1	407	PLUNC	480	TRAIL
43	BMP-15	116	Eotaxin	189	GM-CSF	262	IL-11	335	MCP-2	408	Pref-1	481	TRAIL R1
44	BMPR-IA	117	Eotaxin-2	190	GM-CSF R alpha	263	IL-12 p40	336	MCP-3	409	Progranulin	482	TRAIL R2
45	BMPR-IB	118	Eotaxin-3	191	Granzyme A	264	IL-12 p70	337	MCP-4	410	Prolactin	483	TRAIL R3
46	BMPR-II	119	Epiregulin	192	GREMLIN	265	IL-12 R beta 1	338	M-CSF	411	P-selectin	484	TRAIL R4
47	BTC	120	ErbB2	193	GRO	266	IL-12 R beta 2	339	M-CSF R	412	RAGE	485	TRANCE
48	Cardiotrophin-1	121	ErbB3	194	GRO-a	267	IL-13	340	MDC	413	RANK	486	TREM-1
49	CCL14	122	ErbB4	195	GH	268	IL-13 R alpha 1	341	MFG-E8	414	RANTES	487	TROY
50	CCL28	123	Erythropoietin	196	GHR	269	IL-13 R alpha 2	342	MFRP	415	RELM beta	488	TSG-6
51	CCR1	124	E-Selectin	197	HB-EGF	270	IL-15	343	MICA	416	RELT	489	TSLP R
52	CCR2	125	FADD	198	HCC-4	271	IL-15 R alpha	344	MIF	417	ROBO4	490	TWEAK
53	CCR3	126	FAM3B	199	HCR	272	IL-16	345	MIG	418	S100 A8/A9	491	TWEAK R
54	CCR4	127	Fas	200	Hepassocin	273	IL-17	346	MIP-1a	419	S100A10	492	Ubiquitin+1
55	CCR5	128	Fas Ligand	201	GLO-1	274	IL-17B	347	MIP-1b	420	SAA	493	uPA
56	CCR6	129	FGF Basic	202	HGF	275	IL-17B R	348	MIP-1d	421	SCF	494	uPAR
57	CCR7	130	FGF-BP	203	HGFR	276	IL-17C	349	MIP 2	422	SCF R	495	Vasorin
58	CCR8	131	FGF R3	204	HRG-alpha	277	IL-17D	350	MIP-3 alpha	423	SDF-1	496	VCAM-1
59	CCR9	132	FGF R4	205	HRG-beta 1	278	IL-17E	351	MIP-3 beta	424	sFRP-1	497	VE-Cadherin
60	CD14	133	FGF R5	206	HVEM	279	IL-17F	352	MMP-1	425	sFRP-3	498	VEGF
61	CD27	134	FGF-4	207	I-309	280	IL-17R	353	MMP-2	426	sFRP-4	499	VEGF R2
62	CD30	135	FGF-5	208	ICAM-1	281	IL-17RC	354	MMP-3	427	sgp130	500	VEGF R3
63	CD30 Ligand	136	FGF-6	209	ICAM-2	282	IL-17RD	355	MMP-7	428	SIGIRR	501	VEGF-B
64	CD40	137	FGF-7	210	ICAM-3	283	IL-18 BPa	356	MMP-8	429	Siglec-5	502	VEGF-C
65	CD40 Ligand	138	FGF-8	211	ICAM-5	284	IL-18 R alpha	357	MMP-9	430	Siglec-9	503	VEGF-D
66	CD 163	139	FGF-9	212	IFN-alpha/beta R1	285	IL-18 R beta	358	MMP-10	431	SLPI	504	VEGI
67	Cerberus 1	140	FGF-10	213	IFN-alpha/beta R2	286	IL-19	359	MMP-11	432	Smad 1	505	WIF-1
68	Chem R23	141	FGF-11	214	IFN-beta	287	IL-20	360	MMP-12	433	Smad 4	506	WISP-1
69	Chordin-Like 1	142	FGF-12	215	IFN-gamma	288	IL-20 R alpha	361	MMP-13	434	Smad 5	507	XEDAR
70	Chordin-Like 2	143	FGF-13 1B	216	IFN-gamma R1	289	IL-20 R beta	362	MMP-14	435	Smad 7		
71	CLC	144	FGF-16	217	IGFBP-1	290	IL-21	363	MMP-15	436	Smad 8		
72	CNTF	145	FGF-17	218	IGFBP-2	291	IL-21 R	364	MMP-16	437	Prdx6		
73	CNTF R alpha	146	FGF-18	219	IGFBP-3	292	IL-22	365	MMP-19	438	Soggy-1		

VIII. 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	Reduce sample dilution or concentrate sample
	Improper storage of kit	Store kit at suggested temperature
High background	Excess of biotinylated protein	Make sure to use the correct amount of protein
	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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