

RayBio[®] G-Series Mouse Protein Tyrosine Phosphorylation Antibody Array 1308

For Simultaneously Detecting the Relative Level of Tyrosine
Phosphorylation of Mouse Protein

User Manual

(Revised Oct. 4th, 2022)

Cat#: AAM-PTYR-G1308-4 (4 Sample Kit)

Cat#: AAM-PTYR-G1308-8 (8 Sample Kit)



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RayBiotech Life, Inc.

**RayBio® G-Series Mouse Protein Tyrosine Phosphorylation Antibody
Array 1308 Protocol**

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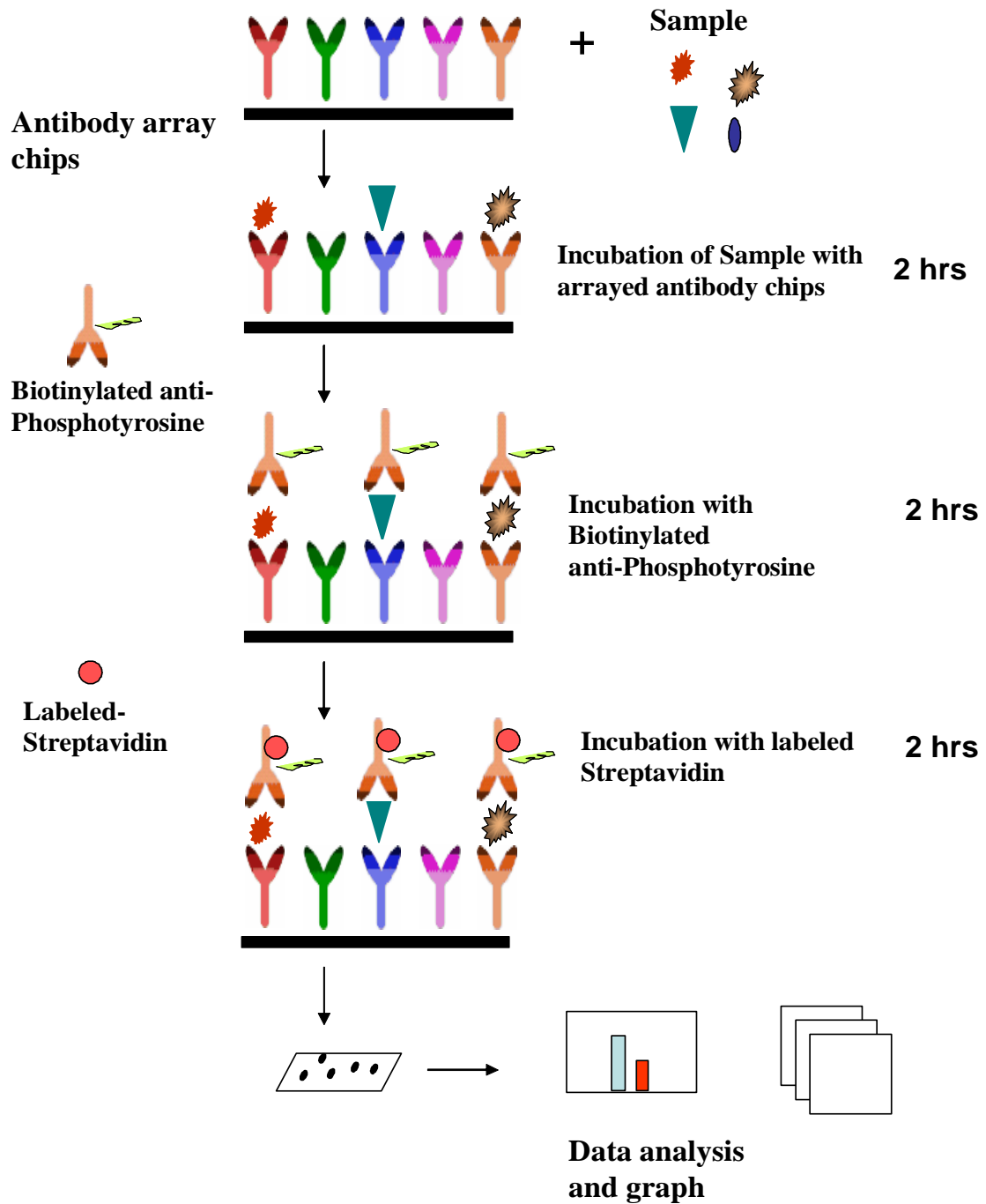
I. Introduction

Protein phosphorylation plays an unusually prominent role in cell signaling, development and growth. The RayBio® G-Series Mouse Protein Tyrosine Phosphorylation Antibody Array 1308 is a very rapid, convenient, and sensitive assay that can simultaneously detect multiple protein phosphorylations and be used to monitor the activation or function of important biological pathways.

RayBiotech is committed to develop a series of phosphorylation antibody arrays. RayBio® Mouse Protein Tyrosine Phosphorylation Antibody Array 1308 is specifically designed for simultaneous identification of the relative levels of phosphorylation of 1308 different Mouse Proteins in cell lysate. By monitoring the changes in protein tyrosine phosphorylation 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.

To use the RayBio® G-Series Mouse Protein Tyrosine Phosphorylation Antibody Array 1308, treated or untreated cell lysate is added into antibody array glass slide wells. The antibody array slide wells are washed, and biotinylated anti-phosphotyrosine antibodies are then used to detect the phosphorylated tyrosines on target proteins. 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.

Here's how it works



II. Materials Provided

Store kit at $\leq -20^{\circ}\text{C}$ immediately upon arrival. Kit must use within the 6 months expiration date.

ITEM	COMPONENT	AAM-PTYR-G1308-4	AAM-PTYR-G1308-8	STORAGE TEMPERATURE AFTER THAWING**
1	RayBio® Glass Slide*	1 slide each of Mouse G1, G2, and G3	2 slides each of Mouse G1, G2, and G3	$\leq -20^{\circ}\text{C}$
2	Blocking Buffer	1 bottle (25ml/ea)	2 bottles (25ml/ea)	
3	Biotinylated Anti-Phosphotyrosine Antibody	3 vials	6 vials	2-8 °C
4	Cy3 equivalent-Conjugated Streptavidin	3 vials	6 vials	2-8 °C
5	20X Wash Buffer I Concentrate	2 bottles (30 ml)	3 bottles (30 ml)	2-8 °C
6	20X Wash Buffer II Concentrate	2 bottles (30 ml)	3 bottles (30 ml)	
7	Wash Buffer III	1 bottle (16 ml)	2 bottles (16 ml)	
8	2X Cell Lysis Buffer Concentrate	1 bottle (10ml)	2 bottles (10ml)	2-8 °C
9	Protease Inhibitor Cocktail	1 vial		$\leq -20^{\circ}\text{C}$
10	Phosphatase Inhibitor Cocktail II	1 vial		
Other Kit Components: Adhesive film				

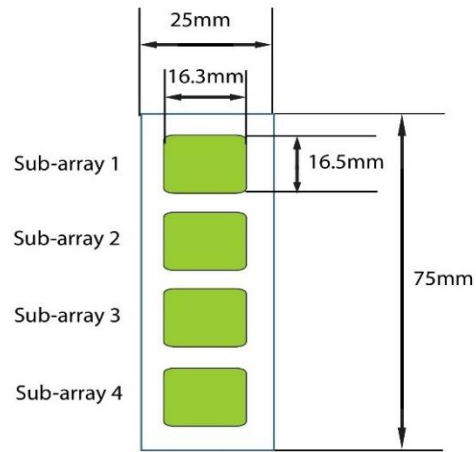
*Each slide contains 4 identical subarrays

**For up to 3 months (unless stated otherwise) or until expiration date

III. Additional Materials Required

- 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 μ l of 1X Cell Lysis Buffer to the vial to prepare a 100X Protease Inhibitor Cocktail Concentrate.
- 2. Phosphatase Inhibitor Cocktail Set II:** Briefly spin down the Phosphatase Inhibitor Cocktail Set II vial before use. Add 180 μ l of 1X Cell Lysis Buffer to the vial to prepare a 25X Phosphatase Inhibitor Cocktail Set II Concentrate. **Dissolve the powder thoroughly by gentle mixing.**
- 3. 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 μ l of the Protease Inhibitor Cocktail Concentrate and 80 μ l of the Phosphatase Inhibitor Cocktail Set II Concentrate into 1.9 ml of the 1X Cell Lysis Buffer to prepare a 1X Cell Lysis Buffer with Protease and Phosphatase Inhibitor Cocktail solution. Mix well before use.
- 4. 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.
- 5. Biotinylated anti-Phosphotyrosine:** Briefly spin down the Detection Antibody vial before use. Add 90 μ l of Blocking Buffer to the vial to prepare a Biotinylated Anti-phosphotyrosine Concentrate. Pipette up and down to mix gently (the Concentrate can be stored at 4 $^{\circ}$ C for 5 days). Add 90 μ l of Detection Antibody Concentrate to a tube with 1710 μ l of Blocking Buffer to prepare a 1X Biotinylated Anti-phosphotyrosine solution. Mix gently.
- 6. Fluorescent dye-Conjugated Streptavidin (Cy3 equivalent):** Briefly spin down the Fluorescent dye-Conjugated Streptavidin vial before use. Add 180 μ l 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. Preparation of Samples

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 2×10^7 cells/ml in the 1X Cell Lysis Buffer with Protease and Phosphatase 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. For incubation with the Phosphorylation Antibody Array G-series 1, use cell lysates at a concentration of 50–1000 µg/ml (as a starting point, we recommend using 400 µg/ml of cell lysate diluted at least 5-fold with the Blocking Buffer).

Lysates should be used immediately or aliquoted and stored at –80 °C. Thawed lysates should be kept on ice prior to use.

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

B. Handling glass slides

- 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. Incubation

- 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.
- Several incubation steps such as step 2 (sample incubation), step 6 (Biotin-conjugated Anti-phosphotyrosine incubation) or step 9 (Fluorescent dye-Conjugated Streptavidin incubation) 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 9 in page 10 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 μ l 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 μ l 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 using 400 μ l of cell lysate at a concentration of 50–1000 μ g/ml (as a starting point, we recommend using 400 μ g/ml cell lysate). **Dilute the lysate at least 5-fold with the Blocking Buffer. 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 μ l of 1X Wash Buffer I at room temperature with gentle shaking.

Note: Avoid the solution overflowing into neighboring wells.

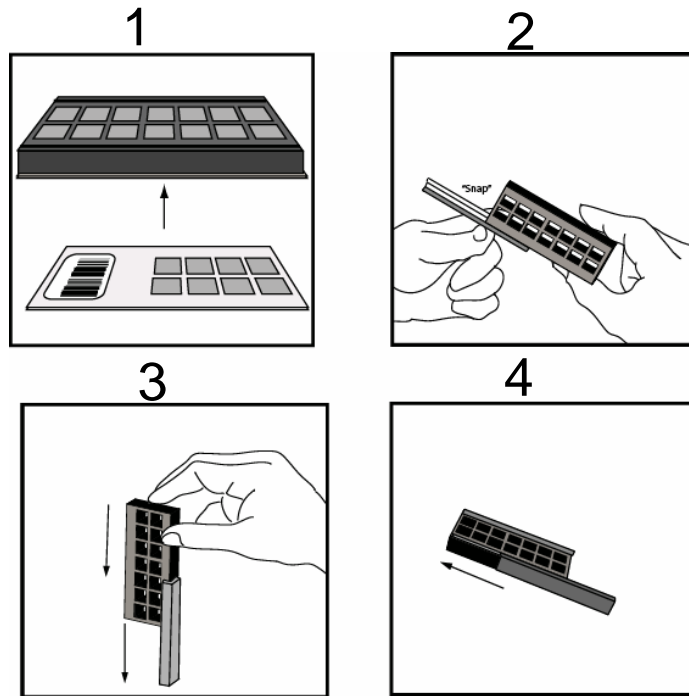
4. Put the Glass Slide with Frame into a box with Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle shaking for 20 min.
5. Decant the Wash Buffer I from each well. Put the Glass Slide with Frame into a box with Wash Buffer II (cover the 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 μ l of the 1X Biotin-conjugated Anti-phosphotyrosine solution to each corresponding well. Incubate at room temperature with gentle shaking for 2 hours.
7. Decant the antibody solution and wash as directed in step 4 three times (wash 3 times, 20 min per wash).
8. Wash as directed in step 5.
9. Remove all of Wash Buffer II from each well. Add 400 μ l 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.

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

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

11. 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).*

12. 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.



13. Wash the glass slide with 40 ml of Wash Buffer II for 5 min. Repeat one more time for a total of 2 washes.
14. 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 N₂ 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

The following figure shows the RayBio® G-Series Mouse Protein Tyrosine Phosphorylation Antibody Array 1 probed with different cell lysates. The images were captured using a laser scanner. 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. Certain proteins containing phosphorylated tyrosine may not be recognized by biotinylated anti-phosphotyrosine because of steric hindrance of the recognition site.

RayBio® Mouse Protein Tyrosine Phosphorylation Antibody Array 1 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	6CKine	63	DPPIV	125	IGFBP-1	187	IL-28B	249	SCF R
2	Activin A	64	DR3	126	IGFBP-2	188	IL-31	250	SDF-1
3	Activin C	65	Dtk	127	IGFBP-3	189	IL-31 RA	251	SAA1
4	Activin R1B	66	EDAR	128	IGFBP-5	190	Insulin	252	Shh-N
5	Adiponectin	67	EGFR	129	IGFBP-6	191	Integrin beta-2	253	SIGIRR
6	AgRP	68	EG-VEGF	130	IGFBP-L1	192	I-TAC	254	SLPI
7	ALCAM	69	Endocan	131	IGF-1	193	GRO alpha	255	Soggy-1
8	ANGPTL2	70	Endoglin	132	IGF-2	194	Kremen-1	256	SPARC
9	ANGPTL3	71	Endostatin	133	IL-1 alpha	195	Kremen-2	257	Spinesin
10	Amphiregulin	72	Eotaxin-1	134	IL-1 beta	196	Lefty-1	258	TACI
11	Artemin	73	Eotaxin-2	135	IL-1 R4	197	Leptin R	259	TARC
12	Axl	74	Epigen	136	IL-1 R6	198	LEPTIN	260	TCA-3
13	bFGF	75	Epregrulin	137	IL-1 R9	199	UF	261	IL-27 R alpha
14	B7-1	76	Erythropoietin	138	IL-1 R1	200	LIGHT	262	TECK
15	BAFF R	77	E-Selectin	139	IL-1 R2	201	LIX	263	TFPI
16	BCMA	78	FADD	140	IL-2	202	LRP-6	264	TGF beta 1
17	beta-Catenin	79	FAM3B	141	IL-2 R alpha	203	L-Selectin	265	TGF beta 2
18	BLC	80	Fas	142	IL-2 R beta	204	Lungkine	266	TGF beta 3
19	Betacellulin	81	Fas Ligand	143	IL-3	205	Lymphotactin	267	TGF beta R1
20	Cardiotrophin-1	82	Fc gamma RIIB	144	IL-3 R alpha	206	LTBR	268	TGF beta R2
21	IL-1ra	83	FGF R3	145	IL-3 R beta	207	MAcCAM-1	269	TSP-1
22	CCL28	84	FGF R4	146	IL-4	208	MCP-1	270	CXCL7
23	MIP-1 beta	85	FGF R5 beta	147	IL-4 R	209	MCP-5	271	Tie-2
24	MCP-3	86	FGF-21	148	IL-5	210	M-CSF	272	TIMP-1
25	MCP-2	87	FIt-3 Ligand	149	IL-5 R alpha	211	MDC	273	TIMP-2
26	CCR10	88	FLRG	150	IL-6	212	MFG-E8	274	TIMP-4
27	CCR3	89	Follistatin-like 1	151	IL-6 R	213	MFRP	275	TL1A
28	CCR4	90	Fractalkine	152	IL-7	214	MIG	276	TLR1
29	CCR6	91	Frizzled-1	153	IL-7 R alpha	215	MIP-1 alpha	277	TLR2
30	CCR7	92	Frizzled-6	154	IL-9	216	MIP-1 gamma	278	TLR3
31	CCR9	93	Frizzled-7	155	IL-9 R	217	MIP-2	279	TLR4
32	CD11b	94	Galectin-3	156	IL-10	218	MIP-3 alpha	280	TMEFF1
33	CD14	95	GCSF	157	IL-10 R alpha	219	MIP-3 beta	281	TNF RI
34	CRP	96	GDF-1	158	IL-11	220	MMP-2	282	TNF RII
35	CD27	97	GDF-3	159	IL-12 p40	221	MMP-3	283	TNF alpha
36	CD27 Ligand	98	GDF-5	160	IL-12 p70	222	MMP-9	284	TNF beta
37	CD30	99	GDF-8	161	IL-12 R beta 1	223	MMP-12	285	Thrombopoietin
38	CD30 Ligand	100	GDF-9	162	IL-13	224	MMP-14	286	TRAIL
39	CD40	101	GFR alpha-2	163	IL-13 R alpha 2	225	MMP-24	287	TRAIL R2
40	CD40 Ligand	102	GFR alpha-3	164	IL-15	226	NRG3	288	TRANCE
41	Cerberus 1	103	GFR alpha-4	165	IL-15 R alpha	227	Neurturin	289	TREM-1
42	Chordin-Like 2	104	GITR	166	IL-16	228	NGFR	290	TROY
43	F3	105	GITR Ligand	167	IL-17A	229	NOV	291	TSLP
44	IL-2 R gamma	106	Glut2	168	IL-17 RB	230	Osteoactivin	292	TSLP R
45	IP-10	107	GM-CSF	169	IL-17C	231	Osteopontin	293	TWEAK
46	Cripto-1	108	Granzyme B	170	IL-17D	232	Osteoprotegerin	294	TWEAK R
47	Crossveinless-2	109	Granzyme D	171	IL-17E	233	OX40 Ligand	295	Ubiquitin+1
48	Cryptic	110	Granzyme G	172	IL-17F	234	PDGF-C	296	uPAR
49	CSK	111	Gremlin-1	173	IL-17 RA	235	PDGF R alpha	297	Urokinase
50	CTACK	112	GHR	174	IL-17 RC	236	PDGF R beta	298	VCAM-1
51	CTLA-4	113	HGFR	175	IL-17 RD	237	Pentraxin-3	299	VE-Cadherin
52	CXCL14	114	HGF	176	IL-18 R alpha	238	PF4	300	VEGF-A
53	CXCL16	115	HVEM	177	IL-20	239	PIGF-2	301	VEGFR1
54	CXCR2	116	ICAM-1	178	IL-20 R alpha	240	Progranulin	302	VEGFR2
55	CXCR3	117	ICAM-2	179	IL-21	241	Prolactin	303	VEGFR3
56	CXCR4	118	ICAM-5	180	IL-21 R	242	P-Selectin	304	VEGF-B
57	CXCR6	119	ICK	181	IL-22	243	RAGE	305	VEGF-C
58	EGF	120	IFN-alpha/beta R1	182	IL-22BP	244	RANTES	306	VEGF-D
59	Decorin	121	IFN-alpha/beta R2	183	IL-23	245	RELM beta	307	WIF-1
60	DKK-1	122	IFN-beta	184	IL-23 R	246	Resistin	308	WISP-1
61	Dkk-3	123	IFN-gamma	185	IL-24	247	S100A10		
62	Dkk-4	124	IFN-gamma R1	186	IL-27	248	SCF		

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 antibodies	Make sure to use the correct amount of antibodies
	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

IX. Reference List

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