

RayBio[®] G-Series Rat Protein Tyrosine Phosphorylation Antibody Array 1500

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

User Manual

(Revised Sept. 12th, 2022)

Cat#: AAR-PTYR-G1500-4 (4 Sample Kit)

Cat#: AAR-PTYR-G1500-8 (8 Sample Kit)



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and Excellent Service**

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RayBiotech Life, Inc.

**RayBio® G-Series Rat Protein Tyrosine Phosphorylation Antibody
Array 1500 Protocol**

TABLE OF CONTENTS

I.	Introduction.....	2
	How It Works.....	3
II.	Materials Provided.....	4
III.	Additional Materials Required.....	5
IV.	Reagent Preparation.....	6
V.	Overview and General Considerations.....	7
	A. Preparation of Samples.....	7
	B. Handling Glass Slides.....	8
	C. Incubation.....	8
VI.	Protocol.....	9
	A. Dry the Array Slides.....	9
	B. Blocking and Incubation.....	9
	C. Fluorescence Detection.....	12
VII.	Interpretation of Results.....	13
VIII.	Troubleshooting Guide.....	16
IX.	Reference List.....	17

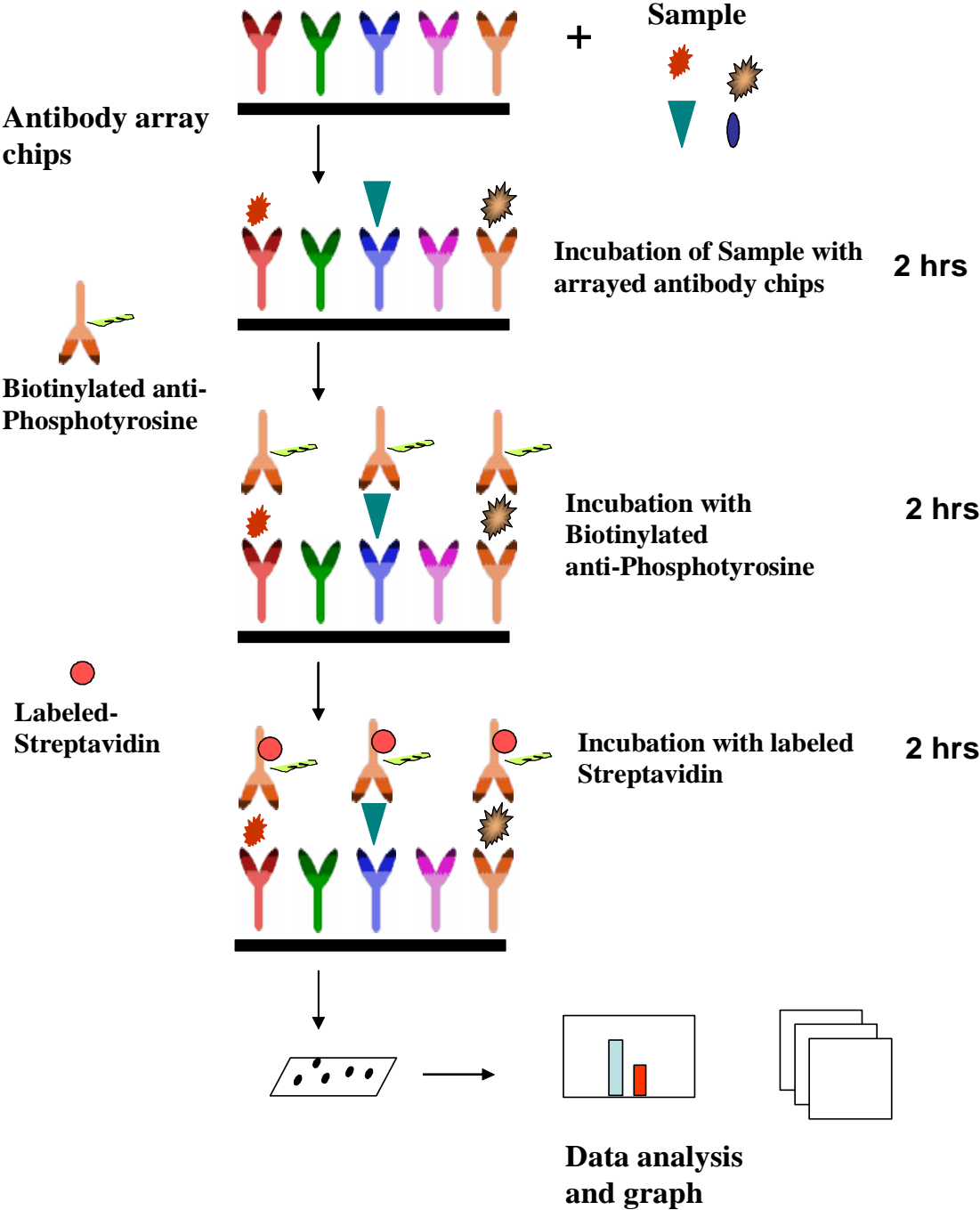
I. Introduction

Protein phosphorylation plays an unusually prominent role in cell signaling, development and growth. The RayBio® G-Series Rat Protein Tyrosine Phosphorylation Antibody Array 1500 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® Rat Protein Tyrosine Phosphorylation Antibody Array 1500 is specifically designed for simultaneous identification of the relative levels of phosphorylation of 1500 different Rat 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 Rat Protein Tyrosine Phosphorylation Antibody Array 1500, 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	AAR-PTYR-G1500-4	AAR-PTYR-G1500-8	STORAGE TEMPERATURE AFTER THAWING**
1	RayBio® Glass Slide*	1 slide each of Rat G2, G3, and G4	2 slides each of Rat G2, G3, and G4	$\leq -20^{\circ}\text{C}$
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 (30ml)	3 bottles (30ml)	2-8 °C
6	20X Wash Buffer II Concentrate	2 bottles (30ml)	3 bottles (30ml)	
7	Wash Buffer III	1 bottle (16ml)	2 bottles (16ml)	
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				

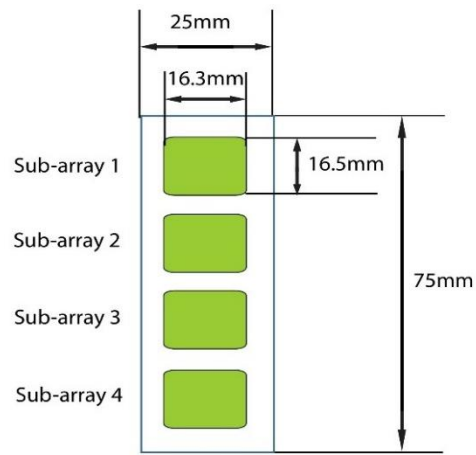
*Each slide contains 4 identical subarrays

**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.

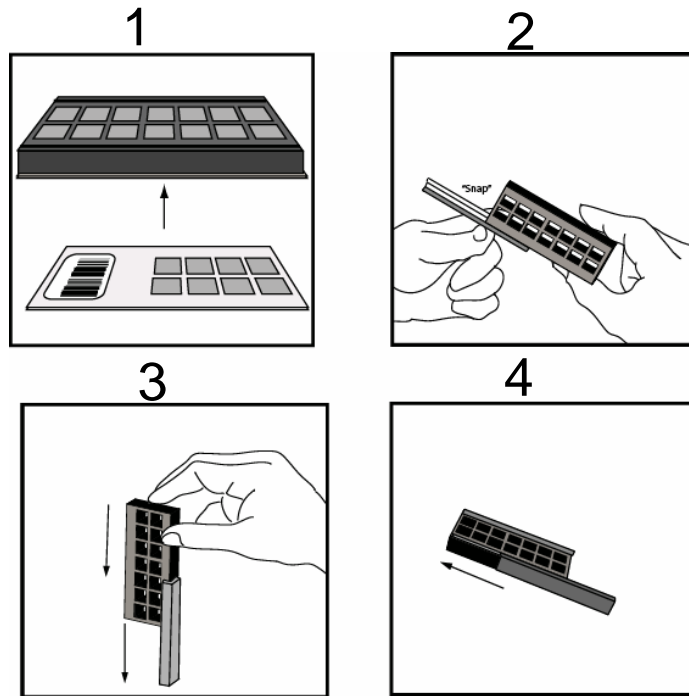
4. Put the Glass Slide with Frame into a box with Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle shaking for 20 min.
5. Decant the Wash Buffer I from each well. Put the Glass Slide with Frame into a box with Wash Buffer II (cover the 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 Rat Protein Tyrosine Phosphorylation Antibody Array 1500 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® G-Series Rat Protein Tyrosine Phosphorylation Antibody Array 1500 Array Map (Rat G2, G3, and G4)

	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
4	42	42	43	43	44	44	45	45	46	46	47	47	48	48	49	49	50	50	51	51	52	52	53	53	54	54	55	55	56	56
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
7	87	87	88	88	89	89	90	90	91	91	92	92	93	93	94	94	95	95	96	96	97	97	98	98	99	99	100	100	101	101
8	102	102	103	103	104	104	105	105	106	106	107	107	108	108	109	109	110	110	111	111	112	112	113	113	114	114	115	115	116	116
9	117	117	118	118	119	119	120	120	121	121	122	122	123	123	124	124	125	125	126	126	127	127	128	128	129	129	130	130	131	131
10	132	132	133	133	134	134	135	135	136	136	137	137	138	138	139	139	140	140	141	141	142	142	143	143	144	144	145	145	146	146
11	147	147	148	148	149	149	150	150	151	151	152	152	153	153	154	154	155	155	156	156	157	157	158	158	159	159	160	160	161	161
12	162	162	163	163	164	164	165	165	166	166	167	167	168	168	169	169	170	170	171	171	172	172	173	173	174	174	175	175	176	176
13	177	177	178	178	179	179	180	180	181	181	182	182	183	183	184	184	185	185	186	186	187	187	188	188	189	189	190	190	191	191
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17	237	237	238	238	239	239	240	240	241	241	242	242	243	243	244	244	245	245	246	246	247	247	248	248	249	249	250	250	251	251
18	252	252	253	253	254	254	255	255	256	256	257	257	258	258	259	259	260	260	261	261	262	262	263	263	264	264	265	265	266	266
19	267	267	268	268	269	269	270	270	271	271	272	272	273	273	274	274	275	275	276	276	277	277	278	278	279	279	280	280	281	281
20	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	282	282	283	283	284	284	285	285	286	286	287	287	288	288	289	289	290	290	291	291	292	292
21	293	293	294	294	295	295	296	296	297	297	298	298	299	299	300	300	301	301	302	302	303	303	304	304	305	305	306	306	307	307
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34	488	488	489	489	490	490	491	491	492	492	493	493	494	494	495	495	496	496	497	497	498	498	499	499	500	500	Neg	Neg	Neg	Neg
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	POS3	POS3	POS2	POS2	POS1	POS1

RayBio® Rat Protein Tyrosine Phosphorylation Antibody Array G2 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	11b-HSD1	73	CD1d1	145	DYRK2	217	GLRX3	289	IPP2	361	Notch-1	433	SEMA4D
2	14-3-3 epsilon	74	CD200	146	DYRK3	218	Glyoxalase 1	290	Islet-1	362	Notch-2	434	SEMA5A
3	14-3-3 eta	75	CD22	147	ECM1	219	Glyoxalase 2	291	Jagged 1	363	Notch-3	435	SEMA7A
4	14-3-3 sigma	76	CD226	148	EEA1	220	Glypican 3	292	JAM-A	364	NPC2	436	Serpin F2
5	14-3-3 theta	77	CD27	149	EFNA1	221	gp130	293	JAM-C	365	NRAGE	437	SerpinA1
6	4-1BB	78	CD276	150	EFNB1	222	GPT	294	JNK1	366	Nrf2	438	SerpinE1
7	A2B5	79	CD300f	151	EGF	223	Gpt2	295	JNK2	367	NRXN1 beta	439	SerpinF1
8	ACACA	80	CD300LG	152	EGFR	224	GPX1	296	KDR	368	Olfactomedin-1	440	SH2B1
9	ACTC1	81	CD31	153	elF5A	225	GPX3	297	Keap1	369	OLR1	441	SHIP2
10	Actin	82	CD34	154	EMP	226	Granzyme B	298	Kirrel3	370	Osteocalcin	442	SHIP-1
11	Activin R2A	83	CD38	155	ENO1	227	GRB2	299	KLKB1	371	OX40	443	SIGNR1
12	ADAM10	84	CD39L1	156	Eotaxin	228	GRIN2A	300	KNG1	372	p27	444	SIRP alpha
13	ADAMTS1	85	CD4	157	EphA5	229	GRK1	301	LAIR1	373	p38 gamma	445	SLAMF1
14	Adiponectin	86	CD47	158	EphB1	230	GRK2	302	LAR	374	p53	446	SLC4A1
15	aFGF	87	CD48	159	EphB6	231	GRK5	303	LAYN	375	p55PIK	447	Slit3
16	Aggrin	88	CD5L	160	Ephrin-A2	232	GRO alpha	304	LDHA	376	PAK1	448	Smad 3
17	AIF	89	CD6	161	Ephrin-B2	233	GRP75	305	Legumain	377	PAK7	449	Smad 7
18	AK1	90	CD63	162	ER alpha	234	HAAO	306	Leptin	378	Pax7	450	SMC1
19	ALCAM	91	CD68	163	ERBB2	235	HABP1	307	Leptin Receptor	379	P-Cadherin	451	Sortilin
20	ALK-7	92	CD79B	164	ERBB3	236	HGF	308	LIF	380	PCDH-17	452	SOST
21	Alpha-Actinin 1	93	CD8 alpha	165	Erythropoietin	237	HIF-1 alpha	309	LIFR	381	PCK1	453	SOX1
22	Alpha-Synuclein	94	CD83	166	Ets-1	238	HO-2	310	LILRA5	382	PDGF-BB	454	SOX10
23	Ameloblastin	95	CD86	167	Ezrin	239	HPRG	311	LILRC2	383	PDGFR-A	455	SOX2
24	AMPK alpha 2	96	CD93	168	F2	240	HPX	312	Lipocalin-2	384	Pentraxin 2	456	SP-D
25	Androgen R	97	CDC25B	169	F3	241	HSP20	313	LMW-PTP	385	Peroxiredoxin 6	457	Src
26	ANGPT1	98	CDC37	170	FABP1	242	HSP27	314	LPHN3	386	PFKM	458	STAT3
27	Annexin A1	99	CDH1	171	FABP2	243	HSP40	315	LRP-4	387	PGC	459	Syndecan-2
28	Annexin A4	100	CDH2	172	FABP3	244	HSP60	316	LTBR	388	plgR	460	Syntaxin 1A
29	Annexin A7	101	CDNF	173	FABP4	245	HSP70	317	LTF	389	PIM2	461	TAFAS
30	Annexin V	102	CES1	174	FABP5	246	HSP90	318	Lyn	390	PKA Ca/b	462	Talin1
31	APE	103	CF XIV	175	FAK	247	HSPA8	319	MAG	391	PKC	463	TCK-1
32	APLP-1	104	CHMP2B	176	FCAR	248	HSPH1	320	Matrilin-3	392	PKC a	464	TC-PTP
33	APRIL	105	Chordin	177	FCGR1	249	HtrA2	321	MBL-2	393	PKC 1/1/z	465	TDP-43
34	Arginase 1	106	CIB1	178	FETUB	250	IDS	322	MCAM	394	PKM2	466	TF
35	ART4	107	CLEC4A2	179	FGF-12	251	IFNA5	323	MCP-3	395	PLAUR	467	TGF-beta RIII
36	ASAH2	108	CLEC4B2	180	FGF-21	252	IFN-alpha	324	MEK2	396	Plexin A4	468	TGM2
37	B3GNT2	109	Clusterin	181	FGFR4	253	IFN-gamma	325	MIF	397	PON3	469	THBD
38	BAFF	110	CNTF	182	Fgr	254	IFN-gamma R2	326	MIG	398	POR	470	Thioredoxin-2
39	BAK	111	CO5	183	Fibromodulin	255	IGF-1	327	MIP-1 alpha	399	PP2A CS	471	TIE-2
40	BCAM	112	COLEC12	184	FKBP12	256	IGFBP-5	328	MIP-3 beta	400	PP2C alpha	472	TIM-1
41	Bcl-10	113	Complexin-2	185	FKBP12.6	257	IGSF8	329	MKK6	401	PPA1	473	TNF alpha
42	Bcl-2	114	Contactin-1	186	FKBP13	258	IKB-beta	330	MMP-2	402	PPP2R4	474	TNF-R1
43	BCL-W	115	Contactin-2	187	FKBP25	259	IKK	331	MMP-8	403	PRDX 2	475	TNFRSF11A
44	Bcl-xL	116	Contactin-4	188	FKBP51	260	IL-1 beta	332	MMP-9	404	PRDX1	476	TNFSF9
45	beta 2-M	117	Contactin	189	FKBP52	261	IL-1 RA	333	MOG	405	PRDX4	477	Tollip
46	beta IG-H3	118	CPA1	190	FLIP	262	IL-10	334	MP1	406	Pref-1	478	TPT1
47	bFGF	119	CPA2	191	FLT1	263	IL-11 R alpha	335	MPO	407	PR1-3	479	TRAF-2
48	BID	120	CPB1	192	Flt-3 Ligand	264	IL-12 p70	336	MST1	408	PRLBA4	480	TRAF-3
49	BIK	121	CRELD1	193	Follistatin	265	IL-13 Ra2	337	NCAM-1	409	PROCR	481	Transgelin
50	BLVRB	122	CRELD2	194	FOLR1	266	IL-15 Ra	338	NCR3	410	Prolactin	482	TREM-1
51	BMP-2	123	CrkL	195	FRK	267	IL-17 RC	339	NEDD4	411	Properdin	483	TRHDE
52	BMP-7	124	CRP	196	FRS2	268	IL-18	340	NEDD8	412	PSAP	484	TrkA
53	B-raf	125	CRYAB	197	GABRA4	269	IL-18 BpC	341	Nephrin	413	PSMA1	485	TrkB
54	BST1	126	CSF1R	198	GAD1	270	IL1R1	342	Nestin	414	PSMA2	486	TrkC
55	BTLA	127	CTACK	199	Galectin-1	271	IL1R2	343	Netrin-1	415	PTK7	487	TWEAK R
56	C4.4A	128	CTGF	200	Galectin-3	272	IL-2	344	Neurexophilin-1	416	PTP1B	488	UCH-L1
57	Cadherin-4	129	CTHRC1	201	Galectin-4	273	IL-2 Ra	345	Neuritin	417	PVR	489	UCH-L3
58	CADM3	130	CTLA4	202	GAPDH	274	IL-2 RG	346	Neurocan	418	PVRL2	490	UNC5H1
59	Calcineurin A	131	Cubilin	203	Gas 1	275	IL-21	347	Neurofascin	419	Ra1A	491	UNC5H2
60	Calcineurin B	132	CXCL10	204	GDF-3	276	IL-22	348	Neurogranin	420	RALT	492	VAMP-2
61	Caspr 2	133	CXCL16	205	GDF-8	277	IL-23 p19	349	Neuroigin-1	421	RANTES	493	VHR
62	Catalase	134	Cyclophilin A	206	GDNF	278	IL-31	350	Neuroigin-2	422	RBBP4	494	Vinculin
63	Cathepsin B	135	Cyclophilin B	207	GFAP	279	IL-4	351	Neuropilin-1	423	RBP4	495	VSIG1
64	Cathepsin C	136	Cystatin C	208	GFRA1	280	IL-4 R	352	Neuroplastin 65	424	Reg III	496	WFDC2
65	Cathepsin E	137	Cytochrome-C	209	GFRA2	281	IL-6	353	NFATC3	425	Reg3B	497	Wnt5a
66	Cathepsin L	138	Decorin	210	GFRA3	282	IL-7	354	NF-L	426	Renin 1	498	XIAP
67	Cathepsin X	139	DEP-1	211	GGT1	283	IL-7 Ra	355	NM23-H1/H2	427	RHD	499	XPNPPE2
68	Caveolin-2	140	DGK-epsilon	212	GH	284	IL-9	356	nNOS	428	ROBO1	500	Zyxin
69	CCK-A R	141	DHFR	213	GIT1	285	IL-9 R	357	NNUP85	429	ROCK2		
70	CCL26-Like	142	Dkk-3	214	GITR	286	ILK	358	Noggin	430	SDC1		
71	CD13	143	DLL1	215	GLA	287	ILKAP	359	Nogo-A	431	Secretagogin		
72	CD14	144	DOK7	216	GLG1	288	IMPDH1	360	Nope	432	SEMA4C		

RayBio® Rat Protein Tyrosine Phosphorylation Antibody Array G3 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	A2M	73	CHGB	145	FGFR5	217	IL-1 R4	289	Neurturin	361	Quiescin Q6	433	TCP1
2	AARE	74	Chk1	146	FGG	218	IL-1 R6	290	Nidogen-1	362	RAGE	434	TDI2
3	ABCF1	75	Chymase	147	FH	219	IL-11	291	Nidogen-2	363	Ras	435	TECK
4	ACAT1	76	CINC-2	148	Fibronectin	220	IL-12 p40	292	NIT2	364	RELN beta	436	Tenascin X
5	Activin A	77	CINC-3	149	Ficolin-2	221	IL-12 RB1	293	NNT	365	Resistin	437	TFF1
6	ADAMTS10	78	Cingulin	150	FLG2	222	IL-13	294	NOV	366	REV3L	438	TFF2
7	ADAMTS15	79	CIP29	151	FOXP3	223	IL-15	295	NPB	367	Rheb	439	TGF-beta 1
8	ADAMTS2	80	Claudin-3	152	Fractalkine	224	IL-16	296	NPTXR	368	RNASE6	440	TGF-beta 2
9	Aggrecan	81	Claudin-4	153	Frizzled-1	225	IL-17A	297	NR3C3	369	ROBO4	441	TGF-beta 3
10	AHCG	82	CNPY2	154	Frizzled-4	226	IL-17C	298	Nrf1	370	ROR1	442	TGF-beta R1
11	AHSG	83	CNTRF	155	Frizzled-5	227	IL-17D	299	OCT3/4	371	RP1	443	TGF-beta R2
12	Akt2	84	COL19A1	156	Frizzled-6	228	IL-19	300	Orexin A	372	RP12	444	TIMP-1
13	Albumin	85	COTL1	157	Frizzled-7	229	IL-2 R beta	301	OSCAR	373	RPL23A	445	TIMP-2
14	AMPKa1	86	CPE	158	FTSL1	230	IL-24	302	OSM	374	RP10	446	Titin
15	ANGPTL2	87	CRADD	159	Galanin	231	IL-27	303	Osteoactivin	375	RP513	447	TK1
16	ANGPTL3	88	CREB	160	GASP-1	232	IL-28 B	304	Osteoadherin	376	RP514	448	TLR1
17	ANKRD9	89	CRF21	161	GASP-2	233	IL-3	305	Osteoprotegerin	377	RPS15A	449	TLR3
18	ANKA6	90	CRHBP	162	G-C-SF R	234	IL-3 R beta	306	p130Cas	378	RP523	450	TLR4
19	APBA2	91	CrkRS	163	GDF-15	235	IL-5	307	p21	379	RPS3A	451	TMEFF1
20	ApoA1	92	CRTAC1	164	GDF-5	236	IMP2	308	P4HB	380	RPS5	452	TMEFF2
21	ApoA2	93	CRTAM	165	GFRA4	237	INSL3	309	Pappalysin-1	381	RPS8	453	TMEM223
22	ApoB	94	CRTH-2	166	GHR	238	Inulin	310	PCAP	382	RP59	454	TOMM70A
23	ApoE	95	Cryptic	167	GKN1	239	I-TAC	311	PCPE-1	383	RREB1	455	TPIS
24	ARHGAP1	96	CSE1L	168	GLI-2	240	Jak2	312	PD-1	384	RSF1	456	TPP1
25	ATG5	97	CSK	169	GLUPR2	241	Kallikrein 10	313	PD-ECGF	385	RUSC1	457	TRADD
26	ATPG	98	CTNND1	170	Glut1	242	Kallikrein 11	314	PDGF-AA	386	S100A10	458	TRAIL R2
27	B3GAT1	99	CXCR2	171	Glut2	243	Kallikrein 5	315	PDGF-C	387	S100A11	459	TRAM
28	B4GalT1	100	CXCR4	172	Glut4	244	Kallikrein 6	316	PDGF-D	388	S100A9	460	TRIM14
29	B7-1	101	CXCR7	173	Glut5	245	KIF5 B	317	PDGFRB	389	S-100b	461	Tropomyosin 3
30	B7-H2	102	Cyclin D1	174	GM2A	246	LAMA5	318	PDLIM5	390	SBP-1	462	TRRAP
31	BAFF R	103	Cyclophilin F	175	GM-CSF	247	LAMP	319	PDZD2	391	SCF	463	Trypsinogen-2
32	Bax	104	Cystatin A	176	GP2	248	LASP1	320	PENK	392	SCF R	464	TSLP
33	BDNF	105	Cystatin B	177	gp340	249	LBP	321	Pentraxin-3	393	SDF4	465	TSP-1
34	beta-NGF	106	Cystatin D	178	GPD1	250	Lefty-1	322	Perilipin-3	394	Septin-7	466	TSP-2
35	BLAME	107	Cystatin E	179	GPR-39	251	Lefty-A	323	Peroxiredoxin-3	395	SERBP1	467	TSP-4
36	BLMH	108	Cystatin S	180	Granzyme A	252	LHPP	324	PF4	396	Serpin A3	468	TTF1
37	BMP-1	109	DAK	181	Granzyme M	253	LIX	325	PFAS	397	Serpin A5	469	TUBA6
38	BMP-15	110	DCI	182	GRHPR	254	LPS	326	PFDN6	398	Serpin B5	470	TWF2
39	BMP-9	111	DCXR	183	GRP	255	LRG1	327	PHGDH	399	Serpin C1	471	TXNDC15
40	BNIP 2	112	DLL4	184	GSK-3 beta	256	LRP-6	328	Piccolo	400	SET	472	TXNDC5
41	BOLA2	113	DMGDH	185	GSN	257	L-Selectin	329	PIK3R2	401	sFRP-4	473	TYRO10
42	BTC	114	DSCAM	186	GSR	258	LUZP1	330	PINCH1	402	SH3BGR13	474	UBC9
43	BTF3	115	DSG1	187	GSTM1	259	Lymphotactin	331	PIP4K2A	403	SHBG	475	Ubiquitin
44	C1q	116	EDA-A2	188	GSTO1	260	MAcCAM-1	332	PLA2G1B	404	SHOX	476	Ubiquitin+1
45	C1s	117	EDAR	189	GULP1	261	MAN1	333	PLD4	405	Siglec-1	477	UNC45A
46	C3a	118	eEF2	190	HAI-1	262	Mcl-1	334	Plexin B2	406	SLC38A10	478	UNC5H4
47	C5a	119	EG-VEGF	191	Haptoglobin	263	MCP-1	335	PIGF-2	407	SUTRK1	479	uPA
48	CA1	120	eIF4E	192	HB-EGF	264	MCP-5	336	PLS3	408	SIP1	480	UROC1
49	CA2	121	EMAP-II	193	HEG1	265	MDC	337	PNP	409	SLURP1	481	USP2
50	CA3	122	Endothelin	194	Hepassocin	266	MEP1A	338	POMC	410	Smad 1	482	Uteroglobin
51	Calbindin D	123	Eotaxin-2	195	HEXB	267	Mesothelin	339	PON1	411	Smad 4	483	VAP-1
52	Cardiotrophin-1	124	EphA1	196	HGFA	268	MICB	340	PP	412	Smad 5	484	VAP-A
53	Cathepsin A	125	EphA2	197	Histone H2AY	269	MIP-3 alpha	341	PPP1CC	413	Smad 8	485	VARS
54	CC128	126	EPHX2	198	hnRNPL	270	MIS RII	342	PRAT4B	414	Somatostatin	486	VDAC1
55	CCR3	127	Epregrulin	199	Hoxb3	271	Mitofusin 2	343	PRELP	415	SOX5	487	VEGF
56	CCR4	128	ERRa	200	HOXD11	272	MKK3	344	Prolactin R	416	SPARC	488	VEGF-B
57	CCT3	129	E-Selectin	201	HSP10	273	MKK4	345	ProSAAS	417	SPINK7	489	VEGF-C
58	CD133	130	EVC2	202	HSP47	274	MMP-10	346	Prostasin	418	SPTBN5	490	VEGFR3
59	CD23	131	Factor IX	203	HTRA1	275	MMP-13	347	Protein Z	419	SSTR2	491	VILIP3
60	CD24	132	Factor V	204	HVEM	276	MMP-16	348	Prouroguanylin	420	STXB2	492	Visfatin
61	CD2AP	133	Factor VII	205	ICAM-1	277	MMP-7	349	PRR4	421	STXEP1	493	Vitronectin
62	CD30	134	Factor XII	206	ICAM-2	278	MRP 1	350	PRRC2A	422	SYK	494	WARS
63	CD40 Ligand	135	FAM3C	207	IDE	279	Multimerin 2	351	PRTN3	423	SYN1	495	WISP-1
64	CD9	136	Fas	208	IFN-beta	280	MySK	352	P-selectin	424	TAC1	496	WISP-2
65	CD90	137	Fas Ligand	209	IFNGR1	281	MyBPC3	353	PSMB1	425	TAGLN2	497	XPB
66	CDC14	138	FGF-11	210	IGFBP-2	282	NACA1	354	PSMD2	426	TALDO	498	XPB
67	CFH	139	FGF-20	211	IGSF4C	283	NADK	355	PSMD9	427	TALDO1	499	YY1
68	CFI	140	FGF-23	212	IL-1 alpha	284	NAGPA	356	PSME1	428	Talin-2	500	ZC3H4
69	CFL1	141	FGF-9	213	IL-1 F10	285	NAPRT1	357	PTHLP	429	TARC		
70	CGA	142	FGF-BP	214	IL-1 F5	286	NeuroD1	358	PTMA	430	TARS		
71	CHCHD3	143	FGFR1	215	IL-1 F6	287	Neurolysin	359	PYY	431	TCA-3		
72	Chemerin	144	FGFR2	216	IL-1 F9	288	Neuropilin-2	360	QARS	432	TcF20		

RayBio® Rat Protein Tyrosine Phosphorylation Antibody Array G4 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	14-3-3 beta	73	CD40	145	FCGR3A	217	IMPAD1	289	NDFP1	361	PNUTS	433	SHIP
2	14-3-3 gamma	74	CD44	146	FCGR2	218	IMPDH2	290	Nectin-3	362	PP2A	434	SHP-2
3	A18G	75	CD51	147	Fen 1	219	Inhibin beta	291	Nesfatin-1	363	PPM1B	435	SIGNR3
4	A1M	76	CD59	148	Filamin A	220	iNOS	292	Nesprin2	364	PPM1L	436	Six3
5	aAmylase	77	CDC25A	149	FKBP38	221	Intellectin-1	293	Neurogenin-2	365	PPP1R9B	437	SMAGP
6	ACE2	78	CDC25C	150	FoxA2	222	IRE1	294	Neuroglycan C	366	PRCP	438	SMOC-1
7	ACLP	79	CDK1	151	FoxP3	223	IRS1	295	NGFR	367	PRDX5	439	SMURF2
8	ACTN2	80	CDK2	152	FPRP	224	IRS2	296	Nicalin	368	PRG2	440	SNAP25
9	ADAM17	81	CEACAM1	153	FSTL4	225	ITGAB	297	Ninjurin-2	369	PRNP	441	SOD1
10	ADAM9	82	CELF1	154	FUCA1	226	ITGB4BP	298	NIPP1	370	Prohibitin	442	SOD2
11	ADNP	83	CES3	155	Fyn	227	ITGB5	299	NKX2.2	371	Prss21	443	SOD-3
12	ADRB2	84	CHORDC1	156	G3BP	228	ITGB6	300	NLRP10	372	PSD-95	444	SPOCK2
13	AFP	85	CKBB	157	G6PD	229	ITPR3	301	NPC1	373	PTEN	445	SQSTM1
14	AGT	86	CLEC1B	158	GABAB R1	230	JAB1	302	NR3C1	374	PTGDS	446	SR-AI
15	Akt1	87	CLEC5A	159	GABAB R2	231	Jak1	303	NrCAM	375	PTGES3	447	ST3GAL2
16	ALDH2	88	COL1A1	160	GABRA1	232	JIP1	304	NSE	376	PTP gamma	448	STAT5b
17	ALOX5	89	COL6A1	161	GABRA5	233	Kallikrein 7	305	NT5E	377	PTP-MEG2	449	STAT6
18	alpha 2u-Globulin	90	COLEC10	162	GALNT2	234	KCNB2	306	NUAK1	378	PTPRM	450	STI1
19	ALPP	91	Complexin-1	163	gamma Catenin	235	KCNC1	307	Nucleostemin	379	PTPRU	451	STIM1
20	AMBP	92	Contactin-3	164	GATE-16	236	KIAA1967	308	NXPH3	380	PVRL1	452	STK3
21	AMH	93	COPZ1	165	GBL	237	Klotho beta	309	Oligodendrocyte Marker O1	381	QDPR	453	Substance P
22	Amphiphysin	94	CPEB3	166	GDF1	238	KMO	310	Oligodendrocyte Marker O4	382	Rab11A	454	SUMO3
23	AMPK beta 1	95	CPM	167	GDF7	239	KOR	311	Oncomodulin	383	Rab27a	455	SUSD2
24	ANG-2	96	CSNK1A	168	GDI1	240	KPNB1	312	OPRM1	384	RAB7A	456	Synaptotagmin-1
25	Angiogenin	97	CSNK1D	169	Gephyrin	241	Kynureninase	313	Osteopontin	385	RAC1	457	Syndecan-3
26	Annexin A11	98	CSNK1E	170	GLUD1	242	LAMC1	314	OV-6	386	RACK1	458	Syntaxin 1B
27	Annexin A2	99	CSNK1G	171	Glycine R	243	Laminin 5	315	P20Sb3	387	Rad17	459	Syntaxin 7
28	ApoH	100	CSNK2B	172	GOLGB1	244	LC3B	316	p38 alpha	388	Ref-1	460	Syntaxin 8
29	ARC	101	CSRFP1	173	GPLD1	245	LHX5	317	p70 S6 Kinase	389	Rap1A/B	461	Syntaxin BP1
30	ATF2	102	CXCR3	174	GPR64	246	LPG	318	PABP	390	Rap2A/B	462	T Cell Receptor alpha Chain-V
31	ATF6	103	CXCR6	175	GPX2	247	Lipin 2	319	PAK4	391	RECQ4	463	TCEB2
32	ATG3	104	CYTL1	176	GPX4	248	LMAN1L	320	PAK6	392	REG4	464	TCP1 eta
33	ATM	105	DARC	177	GRB7	249	LMNA	321	Pannexin-1	393	Relaxin R1	465	Tenascin R
34	Axin-1	106	DARPP-32	178	GRP78	250	LOK	322	Park7	394	RELM gamma	466	Tfr
35	B7-H4	107	DDC	179	GSK-3 alpha	251	LRPAP	323	PARL	395	RGM-B	467	TGN38
36	BAG4	108	DDT	180	H6PD	252	Lumican	324	Parvalbumin	396	RGM-C	468	TH
37	BAG6	109	DDX1	181	HABP2	253	Lysozyme	325	Paxillin	397	RHO G	469	Themis
38	BAMBI	110	DEFA6	182	HAO-1	254	LYVE1	326	PCBP2	398	RIBP	470	Thioredoxin-1
39	BarX1	111	DGK-gamma	183	HBB	255	MAD2L1	327	PCDH12	399	RIPK1	471	Thrombopoietin
40	BCHE	112	DGK-theta	184	HCLS1	256	MafB	328	PCK2	400	RKIP	472	TLR7
41	Bcln1	113	DISC1	185	HDAC2	257	MAP4K4	329	PCNA	401	RNASE4	473	TOP2B
42	beta-Actin	114	Dkk-1	186	HDAC4	258	Matrilin-4	330	PCSK9	402	RNF2	474	TOR
43	beta-I Tubulin	115	Dkk-2	187	HHEX	259	MBP	331	PDAP1	403	ROCK1	475	TRIM63
44	BMX	116	DOCK1	188	HIBADH	260	MCHR1	332	PDCD5	404	RPL10A	476	Troponin T
45	BNIP3L	117	DOT1L	189	HIF-2 alpha	261	M-CSF	333	PDCD6	405	RPL11	477	TRP14
46	BOK	118	DRAK2	190	Histamine H3 R	262	MDGA2	334	PDXH	406	RPL22	478	TRPV1
47	Brevican	119	Draxin	191	Histone H1.3	263	MDH1	335	PDK-1	407	RPLP2	479	TRXR1
48	CA14	120	DSC2	192	Histone H2AX	264	MDM2	336	PDX-1	408	RPS11	480	Trypsin 3
49	Cadherin-15	121	DYRK1A	193	HMGB1	265	MEK1	337	PDK2	409	RPS19	481	Trypsin Pan
50	Cadherin-8	122	Dystroglycan	194	HMGN2	266	MEK2	338	Perilipin-1	410	RPS25	482	TSC22
51	CALD1	123	EDN	195	HMOX1	267	MESDC2	339	PGAM2	411	RPS4X	483	TSH
52	Calretinin	124	EFEMP2	196	HN1	268	Metallothionein	340	PGK1	412	RPS6	484	TXNDC4
53	CaM Kinase II	125	EGLN1	197	hnRNP G	269	mGluR1	341	PGL5	413	RRA2	485	UBASH3B
54	CaMKK alpha	126	EIF3D	198	hnRNP U	270	mGluR2/3	342	PGM1	414	RSK1	486	UBE2N
55	CapG	127	ELAVL1	199	HOMER1	271	mGluR5	343	PGRP-S	415	RSK2	487	UQCERB
56	CART	128	Endoglin	200	HP1BP3	272	MIB1	344	PIK3R1	416	RTN1-A	488	UROD
57	Cathepsin G	129	Endophilin A1	201	HPRT	273	MIOS	345	PIWIL2	417	RYK	489	VAP-B
58	Caveolin-1	130	Endorepellin	202	HS6ST3	274	MIP-1 beta	346	PKA RI beta	418	SC35	490	VE-Cadherin
59	CBP	131	ENSA	203	HSP90B1	275	MKP-3	347	PKC beta 1	419	SCGB3A1	491	Versican
60	CCBL1	132	EpCAM	204	HSPA2	276	MLK4	348	PKC gamma	420	SCG7	492	Vimentin
61	CCR2	133	EphA3	205	HSPB8	277	MN1	349	PKLR	421	SEC13	493	Wnk1
62	CCR6	134	Ephrin-B3	206	IBP160	278	MPP5	350	PKN2	422	SECISBP2	494	Wnk2
63	CCR8	135	ERBB4	207	ICAM-5	279	M-Ras	351	PLA2G2A	423	SEMA3F	495	WT1
64	CCR9	136	ERK1	208	IKK alpha	280	MSH6	352	PLC-beta 4	424	SNP8	496	WWOX
65	CD106	137	ERK2	209	IKK gamma	281	Musashi-1	353	PLC-gamma 1	425	Serpin A12	497	XPB
66	CD161	138	ERK4	210	IL-12 R beta 2	282	MyD88	354	Plexin A1	426	Serpin A3N	498	YAP1
67	CD164	139	FAIM3	211	IL-17F	283	MYHC	355	Plexin A2	427	Serpin A6	499	Yes
68	CD19	140	FANCD2	212	IL17RA	284	Myoglobin	356	Plexin A3	428	Serpin D1	500	ZBTB4
69	CD28	141	Fascin	213	IL-20RB	285	NAP1L1	357	Plexin B3	429	SerpinE2		
70	CD29	142	FASN	214	IL-21R	286	Nbs1	358	PLOD2	430	SerRS		
71	CD36	143	FBPase 1	215	IL-6R	287	NCAM2	359	PLTP	431	SGSH		
72	CD39L4	144	FCGR2B	216	IMPA1	288	NCOR1	360	PNFLA2	432	SHC1		

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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