

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

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

User Manual

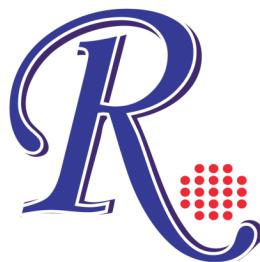
(Revised Oct. 4th, 2022)

**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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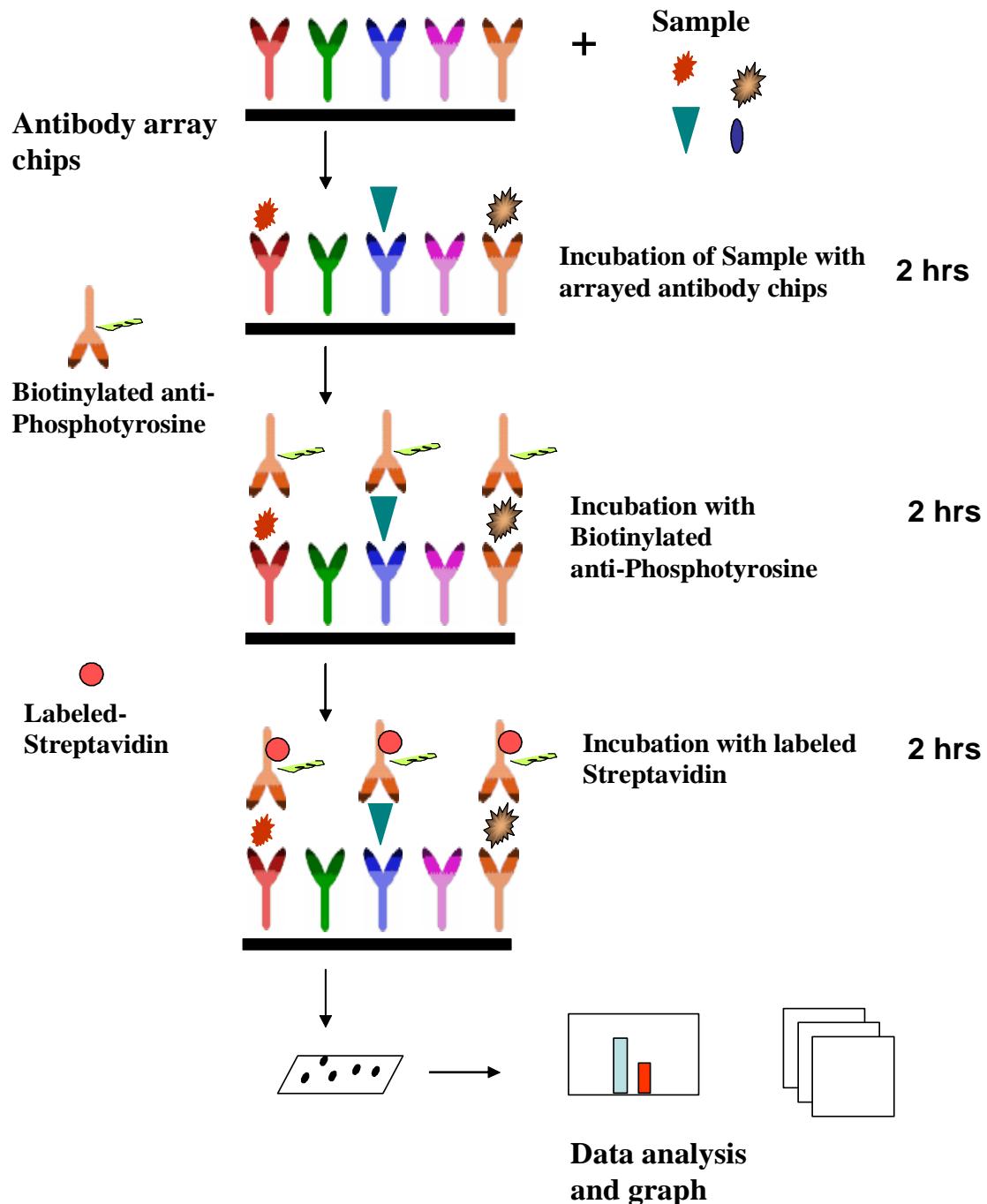
I. Introduction

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 (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					

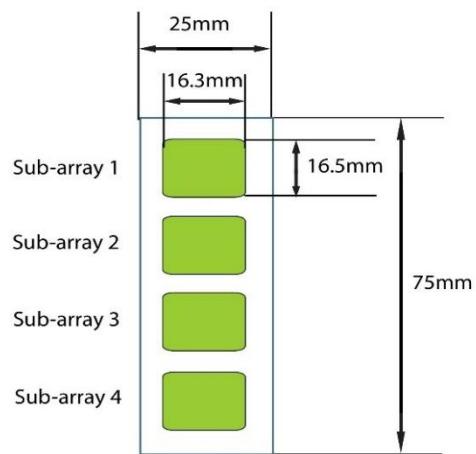
*Each slide contains 4 identical subarrays

**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 µl 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 µl 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 µl of the Protease Inhibitor Cocktail Concentrate and 80 µl 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 µl 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 °C for 5 days). Add 90 µl of Detection Antibody Concentrate to a tube with 1710 µl 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 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. 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 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 using 400 µl of cell lysate at a concentration of 50–1000 µg/ml (as a starting point, we recommend using 400 µg/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.

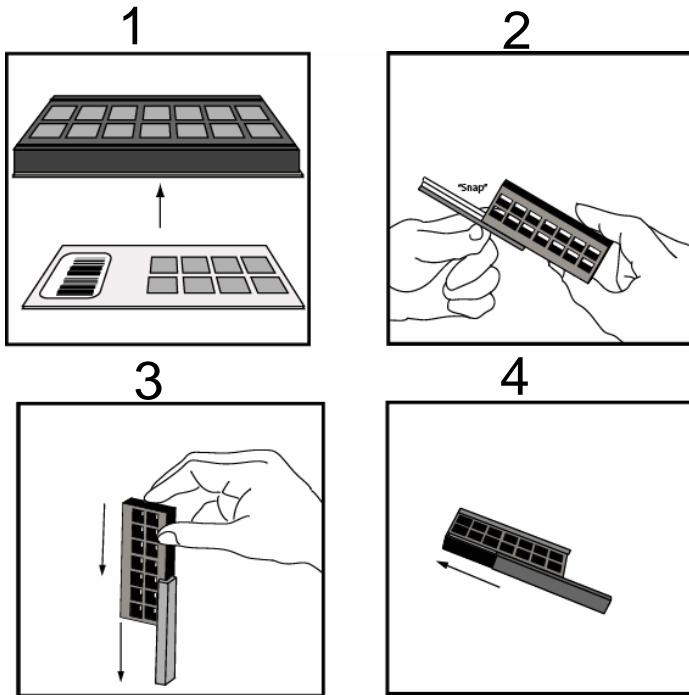
4. Put the Glass Slide with Frame into a box with Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle shaking for 20 min.
5. Decant the Wash Buffer I from each well. Put the Glass Slide with Frame into a box with Wash Buffer II (cover the 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® G-Series Mouse Protein Tyrosine Phosphorylation 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
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
2	11	11	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
3	25	25	26	26	27	27	28	28	29	29	30	30	31	31	32	32	33	33	34	34	35	35	36	36	37	37	38	38
4	39	39	40	40	41	41	42	42	43	43	44	44	45	45	46	46	47	47	48	48	49	49	50	50	51	51	52	52
5	53	53	54	54	55	55	56	56	57	57	58	58	59	59	60	60	61	61	62	62	63	63	64	64	65	65	66	66
6	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80	80
7	81	81	82	82	83	83	84	84	85	85	86	86	87	87	88	88	89	89	90	90	91	91	92	92	93	93	94	94
8	95	95	96	96	97	97	98	98	99	99	100	100	101	101	102	102	103	103	104	104	105	105	106	106	107	107	108	108
9	109	109	110	110	111	111	112	112	113	113	114	114	115	115	116	116	117	117	118	118	119	119	120	120	121	121	122	122
10	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	136	136
11	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
12	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	151	151	152	152	153	153	154	154	155	155	156	156	157	157	158	158	159	159	160	160
13	161	161	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
14	175	175	176	176	177	177	178	178	179	179	180	180	181	181	182	182	183	183	184	184	185	185	186	186	187	187	188	188
15	189	189	190	190	191	191	192	192	193	193	194	194	195	195	196	196	197	197	198	198	199	199	200	200	201	201	202	202
16	203	203	204	204	205	205	206	206	207	207	208	208	209	209	210	210	211	211	212	212	213	213	214	214	215	215	216	216
17	217	217	218	218	219	219	220	220	221	221	222	222	223	223	224	224	225	225	226	226	227	227	228	228	229	229	230	230
18	231	231	232	232	233	233	234	234	235	235	236	236	237	237	238	238	239	239	240	240	241	241	242	242	243	243	244	244
19	245	245	246	246	247	247	248	248	249	249	250	250	251	251	252	252	253	253	254	254	255	255	256	256	257	257	258	258
20	259	259	260	260	261	261	262	262	263	263	264	264	265	265	266	266	267	267	268	268	269	269	270	270	271	271	272	272
21	273	273	274	274	275	275	276	276	277	277	278	278	279	279	280	280	281	281	282	282	283	283	284	284	285	285	286	286
22	287	287	288	288	289	289	290	290	291	291	292	292	293	293	294	294	295	295	296	296	297	297	298	298	299	299	300	300
23	301	301	302	302	303	303	304	304	305	305	306	306	307	307	308	308	Neg	Neg	Neg	Neg	Neg	Neg	POS3	POS3	POS2	POS2	POS1	POS1

RayBio® G-Series Mouse Protein Tyrosine Phosphorylation Antibody Array 2 and 3 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
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
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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
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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</td							

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	Epiregulin	137	IL-1 R9	199	LIF	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	MAdCAM-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	Flt-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	Programulin	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		

RayBio® Mouse Protein Tyrosine Phosphorylation Antibody Array 2 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	14-3-3 beta	73	ASGR2	145	CD21	217	D4	289	Fodrin alpha	361	hnRNP A2B1	433	Lubricin		
2	14-3-3 zeta	74	ASH2L	146	CD39L4	218	DAN	290	Frizzled 8	362	hnRNP C1+C2	434	LUZP1		
3	53BP1	75	ASL	147	CD41	219	DARS2	291	FRY	363	hnRNP G	435	LYZL1		
4	AMY1	76	AspAT	148	CD42b	220	DBH	292	FSH-B	364	hnRNP L	436	MAGI2		
5	AAT1	77	DNPEP	149	CD48	221	DCXR	293	FTL1	365	hnRNP M	437	MAN1		
6	ABAT	78	ASXL1	150	CD5L	222	DDAH1	294	FUCA2	366	hnRNP U	438	MAN1A1		
7	ABCF1	79	ATP5A1	151	CD98	223	DDT	295	FUS	367	Hornerin	439	Mannosidase II		
8	AB13BP	80	ATPB	152	CDA	224	DDX3Y	296	G3BP1	368	Hoxb3	440	MAP1A		
9	ACAA1	81	B3GNT2	153	CDK2	225	DEFA6	297	G6PD	369	HOXD11	441	MAPRE1		
10	ACAA2	82	B4GaIT1	154	CED-6	226	Desmocollin 1	298	GALNT2	370	HP1BP3	442	MARCKS		
11	ACACA	83	B7-H2	155	CENPF	227	Desmocollin-2	299	GANAB	371	HPD	443	MASP3		
12	ACLY	84	BAD	156	CEP57	228	Desmocollin-3	300	GAPDH	372	HPRT1	444	MBD2		
13	ACO1	85	BASP1	157	CES1	229	Desmoglein-1	301	GARNL1	373	HRG	445	MBP		
14	ACTBL2	86	Bassoon	158	Cezanne	230	Desmoglein-2	302	GART	374	HRP12	446	MCAM		
15	ACTC1	87	Bcl2l2	159	CFB	231	Desmoplakin 3	303	Gastrokin 1	375	HSPA1A	447	Mcl-1		
16	ACTG1	88	BCoR	160	CFHR1	232	DGK-theta	304	GATM	376	HTRA1	448	MCM		
17	ACTG2	89	beta I Spectrin	161	CFI	233	DISC 1	305	GBE1	377	HUWE1	449	MDH1		
18	ACTN1	90	beta I Tubulin	162	CFVII	234	DMRN9	306	GCDFP 15	378	IDH1	450	MEP1A		
19	ADA	91	beta III Tubulin	163	Chitobiase	235	DOT1L	307	GCLC	379	IFRD1	451	MT-2		
20	ADAMDEC1	92	BID	164	Chitotriosidase	236	DPP3	308	GCSH	380	IGFBP2	452	Metavinculin		
21	ADAS	93	BIN2	165	Cholinesterase	237	DRIL1	309	GDA	381	IGFBP7	453	MFAP4		
22	ADGRF5	94	Biotinidase	166	CHORDC1	238	DSCAM	310	GDF7	382	IGSF4B	454	MF12		
23	ADGRL4	95	BIRC6	167	CHREBP	239	DSPG3	311	GDI1	383	ILK	455	mGLUR5		
24	ADH1	96	BMP-1	168	Chromogranin B	240	ECHS1	312	GDI2	384	Inhibin beta	456	Mimecan		
25	ADH1C	97	BPGM	169	CKB	241	ECI1	313	Gephyrin	385	Integrin b1	457	MLCK		
26	ADH4	98	BPIFB1	170	CLIC1	242	ECM1	314	GFAP	386	Integrin beta 6	458	MMR		
27	ADH5	99	BPIFB2	171	CLIP1	243	EEF1G	315	GGCT	387	Intergrin a6	459	MN1		
28	ADM	100	Brevican	172	CL-P1	244	EEF2	316	GGH	388	IQGAP2	460	Moesin		
29	Adillin	101	BRG1	173	CLTA	245	EFEMP2	317	GIP	389	IRE1	461	MP1		
30	AEBP1	102	BRSK1	174	CNOT1	246	EFTUD2	318	GLIPR2	390	IRS2	462	MPCA		
31	AFG3L2	103	C10A	175	CO4A2	247	EHD3	319	GLUD1	391	ISOC2	463	MPO		
32	AGA	104	C1QB	176	Cofilin-1	248	Eif4a1	320	Glycoprotein V	392	ITGB4BP	464	MRP 1		
33	Aggrecan	105	C1QR	177	COG4	249	ELAVL1	321	GM2A	393	ITIH2	465	MSH6		
34	Agrin	106	C1RL	178	COL19A1	250	EMSY	322	GMF beta	394	ITIH3	466	Mtor		
35	AGXT	107	C1s	179	COL4A3	251	EN2	323	GNB1	395	ITIH4C	467	Multimerin 2		
36	Ahsp	108	C4BPA	180	Col6A2	252	Endorepellin	324	GNPTG	396	JAM-A	468	MyBPC3		
37	AIFM1	109	C6	181	COL9A3	253	ENO3	325	GOLM4	397	JPT1	469	MYH2		
38	AKAP9	110	C8A	182	COLEC10	254	ENSA	326	GOLM1	398	KDM4B	470	MYH6		
39	AKR1B1	111	C8G	183	Collagen I a1	255	EPB41	327	GPD1	399	Keratin 36	471	MYH7		
40	AKR7A2	112	C9orf40	184	Collagen III	256	EPCR	328	GPLD1	400	KIAAO319L	472	MYHC 2x		
41	ALAD	113	CA1	185	Collagen IVa6	257	Ephrin B1	329	GRHPR	401	KIAA1468	473	MYL12B		
42	ALDH16A1	114	CA150	186	Collagen IX	258	Eps 15	330	GRP170	402	KLKB1	474	MYO5A		
43	ALDH1A1	115	CACNB4	187	Collagen V	259	ERAB	331	GSS	403	KMT2D	475	Myoferlin		
44	ALDH9A1	116	Cadherin 22	188	Collagen X	260	ERP29	332	GSTM1	404	KRT31	476	Myosin 18B		
45	alpha Actinin 4	117	Cadherin-6	189	Collagen XV	261	ERP57	333	GSTO1	405	KRT33B	477	Myosin9		
46	alpha Synuclein	118	CALD1	190	COMP	262	ERP72	334	GSTP1	406	KRT73	478	NABC1		
47	alpha Tubulin 4	119	Calpain S1	191	Corneodesmosin	263	ESD	335	Guanylin	407	KRT82	479	NAGLU		
48	ALPL	120	Calpastatin	192	Cortactin	264	ESR1	336	GZMM	408	KRT85	480	NAP1L1		
49	ALS	121	Calponin-2	193	COTL1	265	Ezrin	337	H6PD	409	KSR1	481	NAPRT1		
50	Alsin	122	Calretinin	194	CPB2	266	FABP5	338	HABP2	410	LAF4	482	NASP		
51	Aminoacylase 1	123	Calumenin	195	CPE	267	Factor IX	339	HBB	411	LAIR1	483	NCAM2		
52	Aminopeptidase A	124	CAP1	196	CPEB3	268	Factor V	340	HDGF	412	LAMB1	484	Nebulin		
53	Androgen Receptor	125	CAPZA1	197	CPM	269	Factor XI	341	Hemoglobin	413	LMNA	485	Nectin-1		
54	ANGPTL6	126	CA2	198	CPNE3	270	Factor XII	342	Hemoglobin A1c	414	LMNB2	486	Nectin-3		
55	ANGPTL8	127	CA3	199	CRHBP	271	Factor XIII	343	HEXB	415	LAMA2	487	Neogenin		
56	Ankrd26	128	Caspase-14	200	Crkl(1)	272	FAH	344	HGFA	416	LAMB2	488	Nesprin2		
57	Annexin A1	129	Catalase	201	CRMP2	273	FAM20C	345	HIBADH	417	LAMC1	489	Neurofibromin		
58	Annexin A2	130	Cathelicidin	202	CRTAC1	274	FAM3C	346	HINT1	418	LAMP1	490	Neurogranin		
59	Annexin A5	131	Cathepsin A	203	CRYZ	275	FASN	347	HIP1R	419	LASP1	491	Neuropeptide B		
60	Annexin A6	132	Cathepsin G	204	Cyclophilin A	276	FASTKD5	348	Histone H1.2	420	LCAT	492	Neuropilin-1		
61	ANP	133	Cathepsin H	205	Cyclophilin B	277	FBP 38	349	Histone H1.4	421	LCMT2	493	Neurotramin		
62	ANP32A	134	Cathepsin Z	206	Cystatin	278	FDPS	350	Histone H2A	422	LDH-H	494	NF-M		
63	Antithrombin III	135	CBS	207	CYT1	279	FGG	351	Histone H2A.Z	423	LEDGF	495	NIF3L1		
64	APIP1	136	CCAR2	208	Cytochrome b5	280	Fibrillin 1	352	Histone H2B K	424	Limbin	496	NME3		
65	AQR	137	CCDC126	209	Cytochrome c	281	Fibrinogen-like 2	353	Histone H3.3	425	LIIM51	497	nNOS1		
66	ARFGEF3	138	CCDC25	210	Cytokeratin 1	282	Fibrinopeptide B	354	Histone H4	426	LMW-PTP	498	Notch-2		
67	Arp3	139	CCS	211	Cytokeratin 10	283	Fibulin 3	355	HMGB1	427	LOK	499	NPAS3		
68	ARPC2	140	CD109	212	Cytokeratin 13	284	Ficolin 2	356	HMGB2	428	LOX	500	NPM1		
69	ARPC3	141	CD133	213	Cytokeratin 14	285	Filamin C	357	HMGB3	429	LOXL1				
70	ARPP19	142	CD148	214	Cytokeratin 15	286	FKBP1A	358	HMGN2	430	LPA				
71	ART3	143	CD155	215	Cytokeratin 20	287	FKBP25	359	HNF-3 alpha	431	LSAMP				
72	ART51	144	CD157	216	Cytokeratin 9	288	FKBP51	360	hnRNP A1	432	LTBP4				

RayBio® Mouse Protein Tyrosine Phosphorylation Antibody Array 3 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	AARE	73	Filaggrin	145	PABP1	217	PREP	289	RPL22	361	SIM2	433	TRAP1
2	ACAT1	74	FITM1	146	PACS1	218	PRG2	290	RPL23A	362	SIRPB1	434	TRAP220
3	ACOT2	75	GARS	147	PNLIP	219	PrP	291	RPL3	363	Six3	435	TRF2
4	ADAM28	76	GCC2	148	PARVB	220	Profilin 1	292	RPL32	364	SLC4A1	436	TRIM14
5	AHCY	77	GLI-2	149	PCAP	221	Prolargin	293	RPL4	365	SLTRK1	437	Tropomyosin 3
6	AK1	78	GLOD4	150	PCBP1	222	Prosaposin	294	RPL7	366	SLURP1	438	TRP-1
7	AKR1A1	79	GLUL	151	PCBP2	223	PTGDS	295	RPL7A	367	SMAD6	439	TRPS1
8	ALDH2	80	GMPR1	152	PCCA	224	PSMD2	296	RPLP0	368	SMC4	440	Trypsinogen-2
9	DEFAS	81	GOLGA3	153	PCDH12	225	Protein C	297	RPLP2	369	SMPD4	441	TSR2
10	ANKRD9	82	GP2	154	PCDH8	226	Protein Z	298	RPS10	370	SNRPD1	442	TTC3
11	ANXA3	83	gp340	155	PCK2	227	PRR4	299	RPS11	371	SOD1	443	TTF1
12	AP180	84	GTF2F1	156	PCMT1	228	PRRC2A	300	RPS12	372	SOD2	444	TUBA6
13	AP352	85	HA1	157	PCNA	229	PRSS23	301	RPS13	373	SOD-3	445	TWF2
14	APLP2	86	HARS	158	PCPE-1	230	PRSS3	302	RPS14	374	Somatotiberin	446	TXND15
15	ApoA V	87	HIC1	159	PCSK9	231	PRTN3	303	RPS15A	375	Somatostatin	447	TXND4
16	ASPM	88	HIP55	160	PDAP1	232	PSMA1	304	RPS16	376	SORD	448	TXND5
17	ASS1	89	H1FO	161	PDE1B	233	PSMA2	305	RPS18	377	SorLA	449	TXNRD2
18	ATOX1	90	HIST1H1B	162	PDIA6	234	PSMA4	306	RPS19	378	SOX4	450	UBA1
19	ATPG	91	HIVEP2	163	PDLIM1	235	PSMA5	307	RPS2	379	SOX5	451	UBE2D3
20	AUTS2	92	hnRNP K	164	PDLIM3	236	PSMA6	308	RPS20	380	SP-D	452	Ube2L3
21	BAI2	93	hnRNP R	165	PDZD2	237	PSMB1	309	RPS23	381	Spectrin	453	UBE2N
22	Barx1	94	HNRPNUL2	166	PEBP1	238	PSMB2	310	RPS25	382	SPEN	454	UCH-L1
23	BBS1	95	HNRP3	167	PEBP4	239	PSMB3	311	RPS3	383	SPG48	455	UFM 1
24	UBC9	96	HP1 gamma	168	PENK	240	PSMB4	312	RPS3A	384	SPINK5	456	UGGT
25	BLM	97	Importin 7	169	PEPD	241	PSMB5	313	RPS4X	385	SPS2L	457	CMPK1
26	BOLA2	98	Involucrin	170	perilipin-3	242	PSMB6	314	RPS5	386	SPTBN2	458	UNC13D
27	C10orf58	99	ISLR	171	Perilipin-1	243	PSMB7	315	RPS8	387	SPTLC1	459	UNC45A
28	CACNA1H	100	ITPR2	172	Periostin	244	PSMC3	316	RPS9	388	Src	460	UNC5H4
29	Calpain-2	101	ITPR3	173	Periplakin	245	PSMD1	317	RREB1	389	SSC5D	461	UPB1
30	CaMK2	102	KCNAB3	174	Peroxiredoxin-2	246	PSMD5	318	RSF1	390	STAT3	462	UQCRB
31	CaMK2D	103	LAMA5	175	Peroxiredoxin-3	247	PSMD9	319	RSU1	391	Stathmin 1	463	UQCRH
32	CBL	104	LDB3	176	Peroxiredoxin-1	248	PSME1	320	RUSC1	392	ST1	464	URB
33	CBR1	105	LHPP	177	PFAS	249	PSME2	321	Septin 7	393	STOM	465	URB2
34	CCDC58	106	LIPG	178	PFDN6	250	PTBP1	322	S100A1	394	STXB2	466	UROC1
35	CCT6A	107	MAP4K4	179	PFKL	251	PTEN	323	S100A11	395	SUCLG1	467	UROD
36	CHCHD3	108	MICALL2	180	PGAM1	252	PTGR1	324	S100A7	396	SUMO3	468	Uroguanylin
37	Cingulin	109	MON2	181	PGAM2	253	PTK7	325	S100A9	397	SVEP1	469	URP2
38	CIT	110	MPST	182	PGK-1	254	PTMA	326	SCD4	398	Symplekin	470	USP14
39	CMG1	111	MRC2	183	PGLS	255	PTPRG	327	SAA4	399	SynCAM	471	USP2
40	CNBP	112	MSH3	184	PG-M	256	PTPRK	328	SBP-1	400	Synemin	472	USP5
41	CNPY2	113	MTA2	185	PGM1	257	PTPRM	329	SC35	401	SYNPO2L	473	Uteroglobin
42	Coilin	114	MTHFD1	186	PGRPL	258	PTPRZ	330	SCG	402	Syntaxin 7	474	Utrophin
43	COL8A2	115	MUC5B	187	PHGDH	259	PZP	331	SCN3A	403	TAB182	475	VARS
44	COLEC11	116	MVD	188	Piccolo	260	QARS	332	SCP2	404	Talin1	476	VAP-1
45	COPG2	117	Myosin IIB	189	pIgR	261	QDPR	333	SDNSF	405	TARS	477	VAP-A
46	CORO1B	118	NACA1	190	PIK3C2B	262	QPRT	334	SDPR	406	TAX1BP3	478	VCP
47	CPA3	119	NAGPA	191	PIN	263	Quiescin Q6	335	SECISBP2	407	TBCA	479	VDAC1
48	CPI17 alpha	120	NAV2	192	PIP5K2 alpha	264	Rab1A	336	Secretogranin V	408	TCEB2	480	VILIP3
49	CrkRS	121	NFATC4	193	PISD	265	Rab7a	337	Semaphorin 6B	409	Tcf20	481	Vimentin
50	CRLF3	122	NNT	194	PLA2G1B	266	Ran	338	Semaphorin 7A	410	TCF1 delta	482	VNN1
51	CSRP3	123	NPEPPS	195	Plastin 3	267	RanBP1	339	SERBP1	411	TCF1 eta	483	VPS4B
52	CTNNAL1	124	NQO2	196	Plastin L	268	RanGAP1	340	Serpin A11	412	TCF1 theta	484	VSIG4
53	CTNND1	125	NSFL1C	197	PLBD2	269	RAP1B	341	Serpin A7	413	TCTP	485	WDR1
54	Cyclophilin F	126	NUCB1	198	PLD4	270	Rbm15	342	Serpin B3D	414	TDIF2	486	WDR44
55	Cystatin C	127	NUP214	199	Plectin	271	RCL	343	Serpin B6	415	Tenascin C	487	WISP2
56	DCAMKL1	128	OAF	200	Plexin B1	272	RECQL	344	Serpin B8	416	Tenascin XB	488	WNK2
57	Dematin	129	OIT3	201	Plexin B2	273	Reg3A	345	Serpin F2	417	TF2F	489	XPG
58	DIAPH1	130	OPCML	202	POLD1	274	REV3L	346	Serpin H1	418	TGM3	490	YB1
59	DKC1	131	ORM2	203	POLD2	275	RHOC	347	Serpin A10	419	Thioredoxin-1	491	SYN1
60	DLST	132	OSBP1	204	Plxd2c	276	RHOG	348	SERPINB1	420	THOP1	492	YY1
61	DMRT1	133	OSCAR	205	PMCA	277	RNASE1	349	SerpinB4	421	TIF1 alpha	493	ZAK
62	Dystrophin	134	OSM R beta	206	PNP	278	RNASET2A	350	SerpinE2	422	TMEM103	494	zbtb11
63	Ebf4	135	Osteoadherin	207	POLD2	279	RLF	351	SerRS	423	TOB2	495	ZBTB4
64	EBP50	136	OTC	208	POLR2A	280	RNAE4	352	SET	424	TOMM70A	496	ZC3H18
65	ECHDC1	137	OTUB1	209	POR	281	Rnose2	353	SEZ6L2	425	TOP2B	497	ZC3H4
66	EHHADH	138	OTUD7A	210	PPOX	282	RP1	354	SF20	426	TPD52L2	498	ZC3H8
67	EIF3D	139	OT-NPI	211	PPP1CC	283	RPL10	355	SHANK1	427	TPM4	499	ZNF295
68	EIF4A2	140	p16 ARC	212	PPP1R9A	284	RPL10A	356	SHC1	428	TPP1	500	Zyxin
69	EIF4GII	141	p23	213	PPP2R1B	285	RPL11	357	SHMT1	429	TPPP3		
70	ENDOD1	142	p39	214	PPP2R4	286	RPL12	358	SHOX	430	TPR		
71	EYA2	143	P4HB	215	PRCP	287	RPL14	359	SHP-1	431	TALDO1		
72	F8	144	p73	216	PRDM13	288	RPL17	360	Siglec-1	432	Transthyretin		

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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RayBio® Cytokine Antibody Arrays are patent-pending technology developed by RayBiotech.

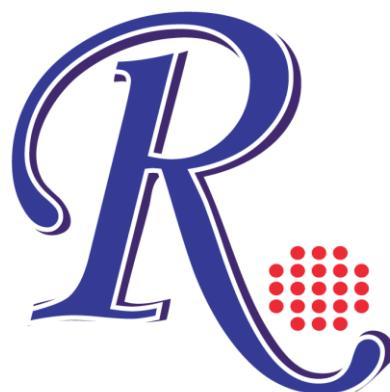
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