RayBio® G-Series Rat Protein Tyrosine Phosphorylation Antibody Array 2

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

User Manual

(Revised Mar. 20th, 2024)

Cat#: AAR-PTYR-G2-4 (4 Sample Kit)
Cat#: AAR-PTYR-G2-8 (8 Sample Kit)

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Empowering your proteomics

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RayBiotech Life, Inc.

RayBio® G-Series Rat Protein Tyrosine Phosphorylation Antibody Array 2 Protocol

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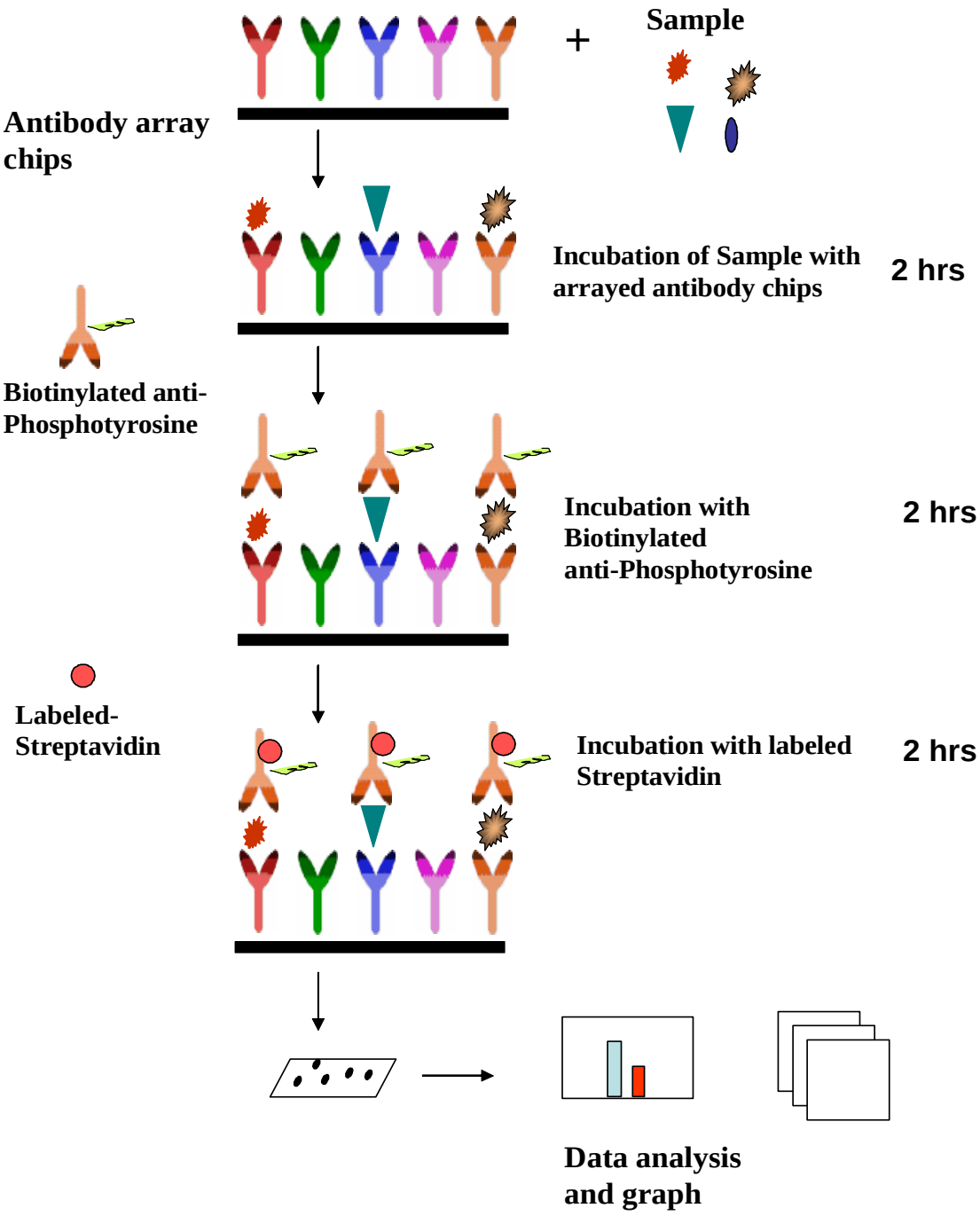
I. Introduction

Protein phosphorylation plays an unusually prominent role in cell signaling, development and growth. The RayBio® G-Series Rat Protein Tyrosine Phosphorylation Antibody Array 2 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 2 is specifically designed for simultaneous identification of the relative levels of phosphorylation of 500 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 2, 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 ≤ -20 °C immediately upon arrival. Kit must use within the 6 months expiration date.

ITEM	COMPONENT	AAR-PTYR-G2-4	AAR-PTYR-G2-8	STORAGE TEMPERATURE AFTER THAWING**
1	RayBio® Glass Slide*	1	2	≤-20°C
2	Blocking Buffer	1 bottle (8ml/ea)	2 bottles (8ml/ea)	
3	Biotinylated Anti- PhosphoTyrosine Antibody	1 vial	2 vials	2-8°C
4	Cy3 equivalent-Conjugated Streptavidin	1 vial	2 vials	2-8 °C
5	20X Wash Buffer I Concentrate	1 bottle (30 ml)		2-8 °C
6	20X Wash Buffer II Concentrate	1 bottle (30 ml)		
7	Wash Buffer III	1 bottle (20 ml)		
8	2X Cell Lysis Buffer Concentrate	1 bottle (10ml)		2-8 ℃
9	Protease Inhibitor Cocktail	1 vial		≤-20°C
10	Phosphatase Inhibitor Cocktail II	1 vial		
Other Kit Components: Adhesive film				

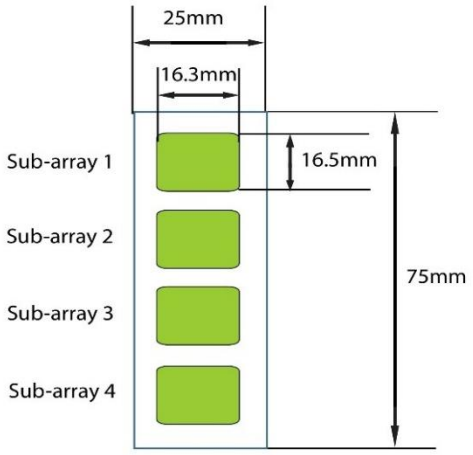
*Each slide contains 4 identical subarrays

**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 ^{0}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 $2x10^7$ 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 ug/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 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 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.

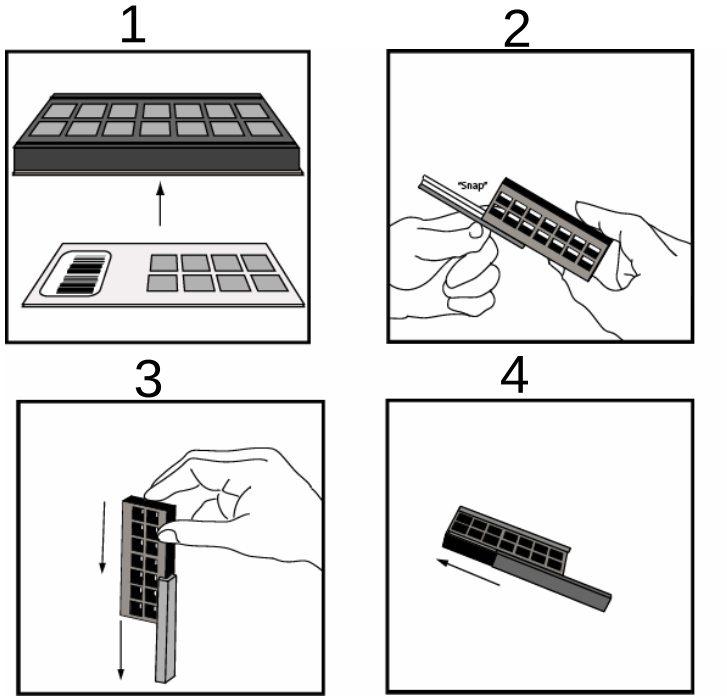
9. Remove all of Wash Buffer II from each well. Add 400 µl of the 1X Fluorescent dye-Conjugated Streptavidin solution to each subarray. Cover the incubation chamber with the Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in a dark room.

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

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 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

The following figure shows the RayBio® G-Series Rat Protein Tyrosine Phosphorylation Antibody Array 2 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 2 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
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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
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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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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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33	473	473	474	474	475	475	476	476	477	477	478	478	479	479	480	480	481	481	482	482	483	483	484	484	485	485	486	486	487	487
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	eIF5A	225	GPX3	297	Keap1	369	OLR1	441	SHIP2
10	Actin	82	CD34	154	EMP	226	Granzyme B	298	Kirrel3	370	Osteocalcin	442	SHP-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	Agrin	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	PDGFRA	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	pIgR	460	Syntaxin 1A
29	Annexin A7	101	CDNF	173	FABP4	245	HSP70	317	LTF	389	PIM2	461	TAFA5
30	Annexin V	102	CES1	174	FABP5	246	HSP90	318	Lyn	390	PKA C a/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 i/l/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	Cortactin	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	PRL-3	479	TRAF-2
48	BID	120	CPB1	192	Flt-3 Ligand	264	IL-12 p70	336	MST1	408	PRL8A4	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	RalA	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	Neuroligin-1	421	RANTES	493	VHR
62	Catalase	134	Cyclophilin A	206	GDNF	278	IL-31	350	Neuroligin-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	XPNPEP2
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		

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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