RayBio® G-Series Rat Protein Tyrosine Phosphorylation Antibody Array 4

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

User Manual

(Revised Mar. 20th, 2024)

Cat#: AAR-PTYR-G4-4 (4 Sample Kit)
Cat#: AAR-PTYR-G4-8 (8 Sample Kit)

RayBiotech
Empowering your proteomics

We Provide You with Quality Products and Excellent Service

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RayBiotech Life, Inc.

RayBio® G-Series Rat Protein Tyrosine Phosphorylation Antibody Array 4 Protocol

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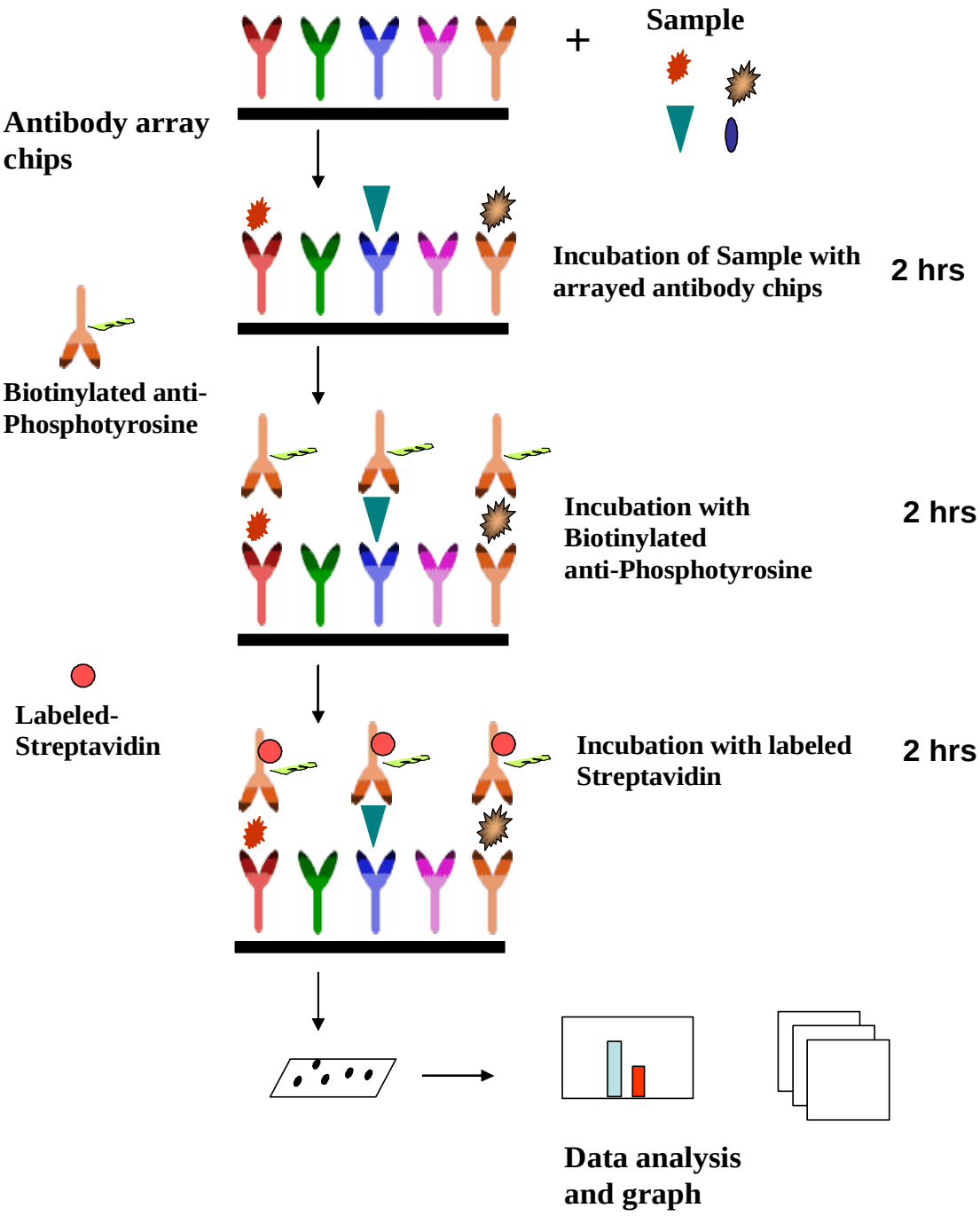
I. Introduction

Protein phosphorylation plays an unusually prominent role in cell signaling, development and growth. The RayBio® G-Series Rat Protein Tyrosine Phosphorylation Antibody Array 4 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 4 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 4, 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-G4-4	AAR-PTYR-G4-8	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				

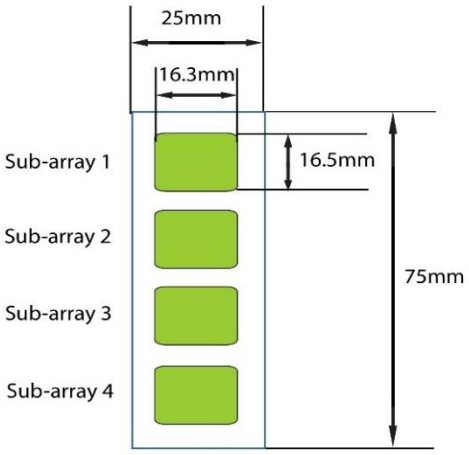
*Each slide contains 4 identical subarrays

**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.

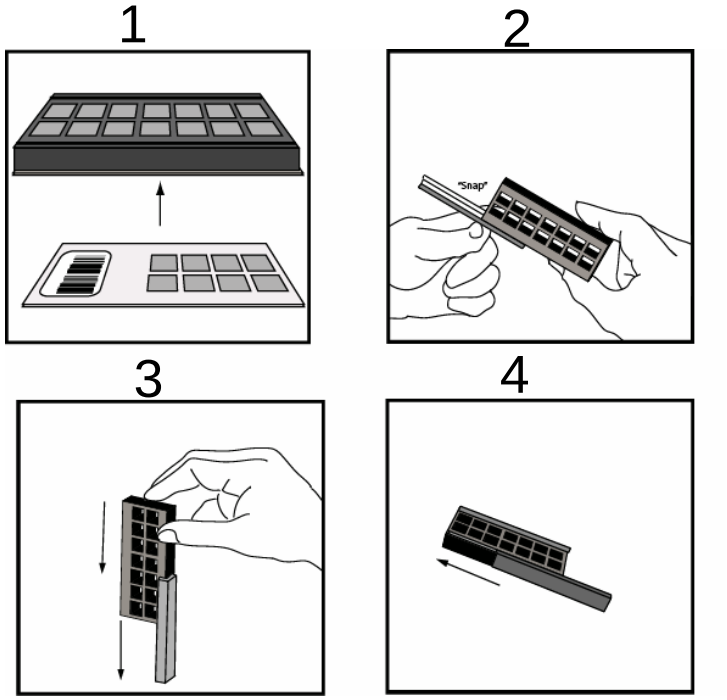
9. Remove all of Wash Buffer II from each well. Add 400 µl of the 1X Fluorescent dye-Conjugated Streptavidin solution to each subarray. Cover the incubation chamber with the Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in a dark room.

   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 4 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 4 Array Map

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11
2	12	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26
3	27	27	28	28	29	29	30	30	31	31	32	32	33	33	34	34	35	35	36	36	37	37	38	38	39	39	40	40	41	41
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5	57	57	58	58	59	59	60	60	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70	71	71
6	72	72	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84	85	85	86	86
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
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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
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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 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	NDFIP1	361	PNUTS	433	SHIP
2	14-3-3 gamma	74	CD44	146	FCGRT	218	IMPDH2	290	Nectin-3	362	PP2A	434	SHP-2
3	A1BG	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	Intelectin-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	ITGA8	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	GABABR1	230	JAB1	302	NR3C1	374	PTGDS	446	SR-AI
15	Akt1	87	CLEC5A	159	GABABR2	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 S	315	P2OSb3	387	Rad17	459	Syntaxin 7
28	ApoH	100	CSNK2B	172	GOLGB1	244	LC3B	316	p38 alpha	388	Raf-1	460	Syntaxin 8
29	ARC	101	CSRP1	173	GPLD1	245	LHX5	317	p70 S6 Kinase	389	Rap1A/B	461	Syntaxin BP1
30	ATF2	102	CXCR3	174	GPR64	246	LIPG	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	RHOG	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	Beclin 1	113	DISC 1	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	PDHX	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	PDZK1	409	RPS19	481	Trypsin Pan
50	Cadherin-8	122	Dystroglycan	194	HMGN2	266	MEKK2	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	PGLS	413	RRAS2	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	UQCRB
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	SCGF	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	SENP8	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	PNPLA2	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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