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

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

User Manual

(Revised Mar. 20th, 2024)

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

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Empowering your proteomics

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Tel: (770) 729-2992 **Fax: (888) 547-0580**
Web: www.raybiotech.com **Email: info@raybiotech.com**

RayBiotech Life, Inc.

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

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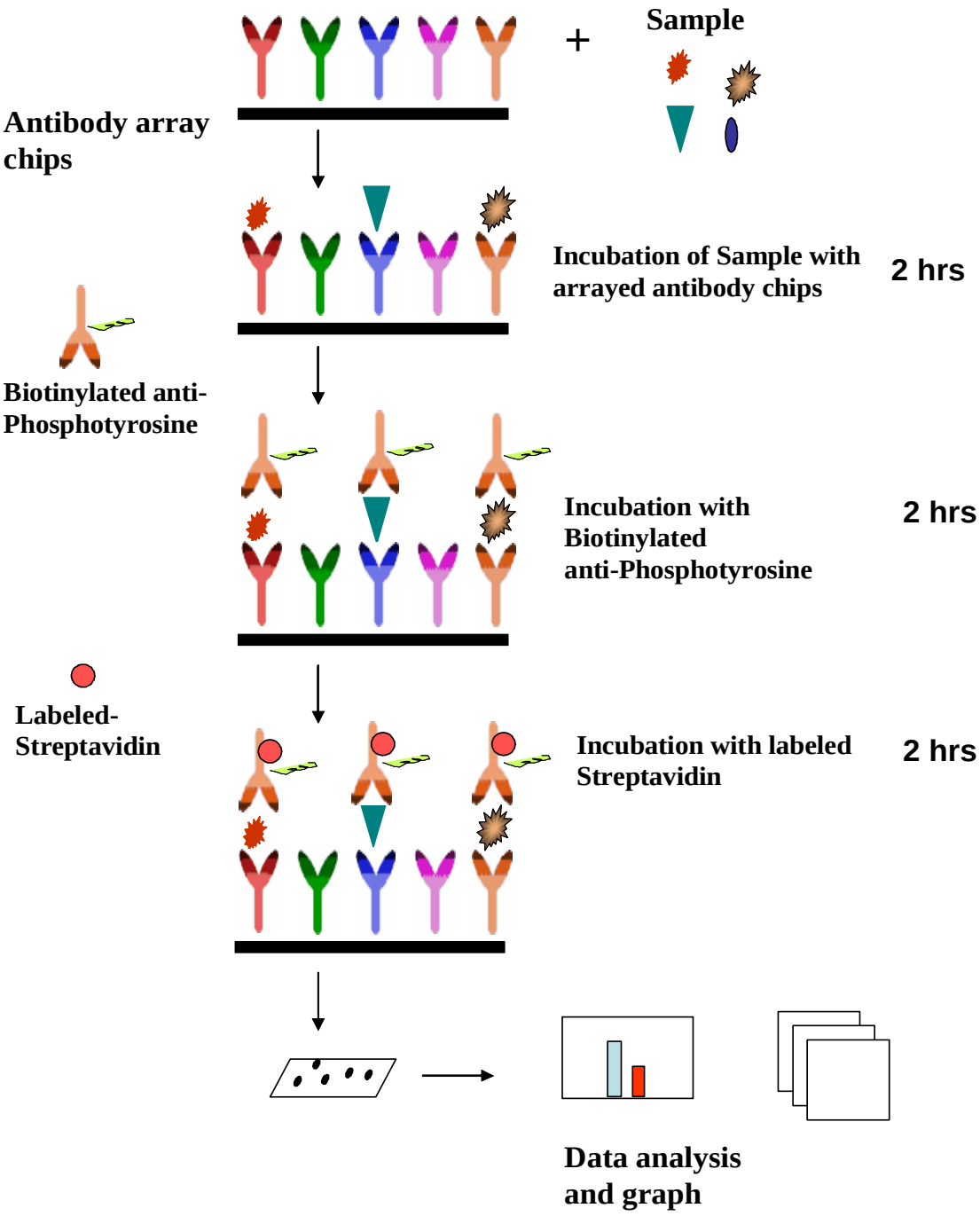
I. Introduction

Protein phosphorylation plays an unusually prominent role in cell signaling, development and growth. The RayBio® G-Series Human 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® Human Protein Tyrosine Phosphorylation Antibody Array 4 is specifically designed for simultaneous identification of the relative levels of phosphorylation of 500 different Human 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 Human 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

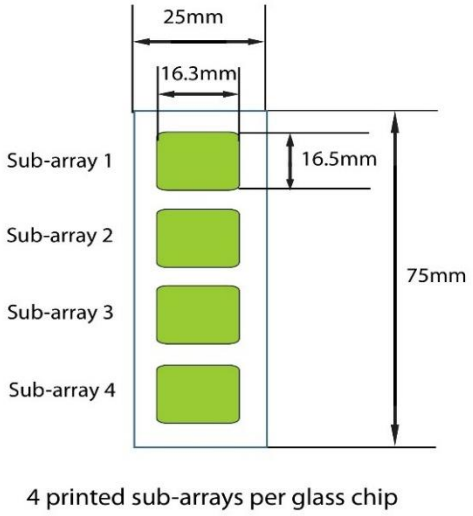
Upon receipt, the kit should be stored at -20 °C to -80 °C. Please use within 6 months from the date of shipment. After initial use, the 2X Cell Lysis Buffer, Blocking Buffer, 20X Wash Buffer I, 20X Wash Buffer II, Biotin-Conjugated Anti-phosphotyrosine and Fluorescent dye-Conjugated Streptavidin (Cy3 equivalent) should be stored at 4 °C to avoid repeated freeze-thaw cycles. The Array I Glass Slide, Protease Inhibitor Cocktail and Phosphatase Inhibitor Cocktail Set II should be kept at -20 °C to -80 °C. Use within 3 months after initial use.

- RayBio® G-Series Human Protein Tyrosine Phosphorylation Antibody Array 4 Glass Slide with Frame (each slide contains 4 Subarrays, with 1 slide included for AAH-PTYR-G4-4 (4 Sample Kit), and 2 slides included for AAH-PTYR-G4-8 (8 Sample Kit))
- 2X Cell Lysis Buffer (10 ml)
- Protease Inhibitor Cocktail (1 or 2 tubes, 1 tube included for the 4 Sample Kit, and 2 for the 8 Sample Kit)
- Phosphatase Inhibitor Cocktail Set II (1 or 2 tubes, 1 tube included for the 4 Sample Kit, and 2 for the 8 Sample Kit)
- Blocking Buffer (8 ml, 1 or 2 bottles, 1 bottle included for the 4 Sample Kit, and 2 for the 8 Sample Kit)
- 20X Wash Buffer I (30 ml)
- 20X Wash Buffer II (30 ml)
- Biotin-Conjugated Anti-phosphotyrosine (1 or 2 tubes, 1 tube included for the 4 Sample Kit, and 2 for the 8 Sample Kit)
- Fluorescent dye-Conjugated Streptavidin (Cy3 equivalent) (1 or 2 tubes, 1 tube included for the 4 Sample Kit, and 2 for the 8 Sample Kit)
- Wash Buffer III (20 ml)
- Adhesive film

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.

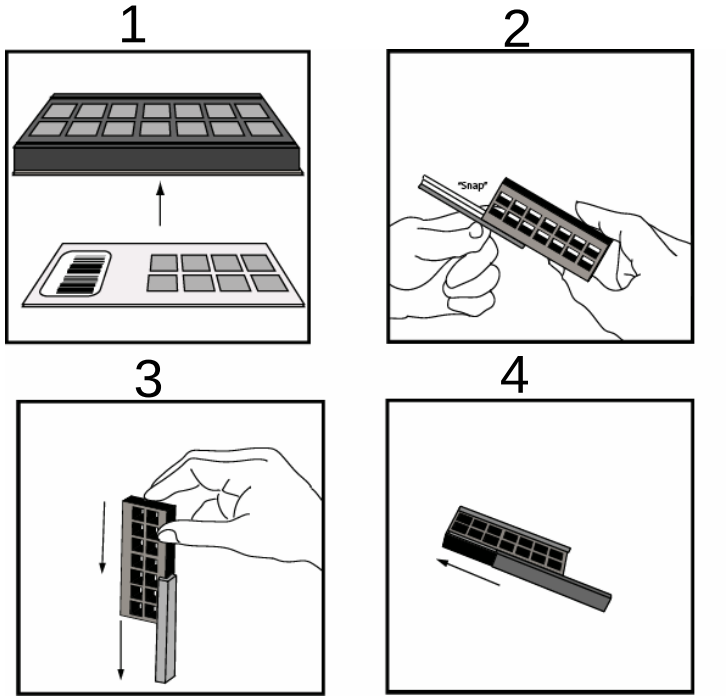
9. Remove all of Wash Buffer II from each well. Add 400 µl of the 1X Fluorescent dye-Conjugated Streptavidin solution to each subarray. Cover the incubation chamber with the Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in a dark room.

   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 Human 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 Human 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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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® G-Series Human Protein Tyrosine Phosphorylation Antibody Array 4 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	HEXA	73	LIMS1	145	Nectin-1	217	Peroxiredoxin 3	289	PTK 7	361	Serpin A7	433	Thymosin b10
2	HTRA1	74	LMAN2	146	Nectin-3	218	Peroxiredoxin 5	290	PTMA	362	Serpin B3	434	Titin
3	Agrin	75	ACP1	147	NEDD8	219	PF4V1	291	PTP gamma	363	Serpin B6	435	TLS
4	IBP160	76	LOK	148	Neogenin	220	PGAM1	292	PTP kappa	364	Serpin B8	436	TMEM223
5	IDH1	77	LOX	149	Nesprin2	221	PGAM2	293	PTP mu	365	Serpin F2	437	TOB2
6	IDH3A	78	LOXL1	150	Neurabin 1	222	PGD	294	PTPRS	366	Serpin A10	438	TOP2B
7	IFRD1	79	LRP 4	151	Neural Cadherin	223	PHGDH	295	PTPRZ	367	SERPINB1	439	TPM4
8	IGF2BP2	80	LTA4H	152	PAM	224	PGK-1	296	PYGL	368	SerpinB4	440	TPP1
9	ITGB5	81	LTBP4	153	Neurogranin	225	PGLS-C-t	297	PZP	369	SerpinE2	441	TALDO1
10	IGSF4B	82	Lubricin	154	Neuropeptide B	226	PGM1	298	QDPR	370	SerRS	442	TALDO
11	Ihh	83	LUZP1	155	Neuropilin-1	227	PGRPL	299	QPRT	371	SET	443	Transthyretin
12	ILK	84	LYPA1	156	Neurotrimin	228	PHAP1	300	Quiescin Q6	372	SEZ6L2	444	TRAP1
13	Inhibin beta	85	Lysozyme	157	NF-M	229	PSAT1	301	Rab7a	373	SF20	445	TRAP220
14	ITGB1	86	MAGI2	158	Nidogen-2	230	PIK3C2B	302	Ran	374	SH3BGRL	446	TRF 2
15	ITGB6	87	MAGP-2	159	NIT2	231	pIgR	303	RanGAP1	375	SH3BGRL3	447	TPIS
16	ITGA6	88	MAN1	160	NME3	232	PIK3IP1	304	RAP1AB	376	SHANK1	448	Tropomyosin 3
17	IQGAP1	89	MANF	161	nNOS	233	PIN	305	Rbm15	377	SHC1	449	Twist-1
18	IQGAP2	90	Mannosidase II	162	Noelin	234	PISD	306	RCL	378	SHIP	450	TRPS1
19	IRE1	91	MAP1A	163	Non-muscle Actin	235	PKLR	307	Reg1A	379	SHMT1	451	Trypsinogen-2
20	IRS2	92	MAPRE1	164	Myosin IIA	236	PLA2G1B	308	Reg3A	380	SHP-1	452	Trypsin Pan
21	ISOC2	93	MARCKS	165	Notch-2	237	Plakophilin 1	309	RHOC	381	Siglec-1	453	WRS
22	ITGB4BP	94	MASP3	166	Notch-2 ICD	238	Plastin L	310	RhoGDI	382	SIGLEC14	454	TSR2
23	ITIH1	95	MBD2	167	NPAS3	239	PLC-gamma 1	311	RNASE1	383	SIM2	455	TUBA6
24	ITIH2	96	MBP	168	NPM1	240	Pleckstrin	312	RNH1	384	SIRP beta 1	456	TWF2
25	ITIH3	97	MCAM	169	NQO2	241	Plectin	313	RNASET2	385	Six3	457	TXNDC4
26	ITIH4 a	98	Mcl-1	170	NT5C3	242	Plexin B1	314	RKIP	386	SLC38A10	458	TXNDC5
27	JAM-A	99	MCM	171	NUCB1	243	Plexin B2	315	POLR2A	387	SLITRK1	459	TXNRD2
28	JARID2	100	MCM5	172	NUP98	244	PLOD1	316	RNASE4	388	SLURP1	460	UBE2D3
29	KPNB1	101	MCMP2	173	OBCAM	245	PLOD2	317	RNASE6	389	SMA	461	Ube2L3
30	Keratin 36	102	MDH1	174	OIT3	246	PLS3	318	RPL10	390	SMC4	462	UBE2N
31	Keratin 38	103	MDH2	175	Olfactomedin-2	247	Plxdc2	319	RPL10A	391	SMPD4	463	Ubiquitin
32	KHSRP	104	ME1	176	OTC	248	PNP	320	RPL11	392	SOD1	464	UCH-L1
33	KIAA0319L	105	MEP1A	177	Orosomucoid 2	249	POR	321	RPL12	393	SOD2	465	UFM 1
34	KIAA1468	106	Metallothionein	178	ORP150	250	PPCS	322	RPL14	394	SOD-3	466	UGGT
35	KIAA1967	107	Metavinculin	179	OSBP1	251	PPOX	323	RPL17	395	SOD4	467	UNC13D
36	KIF5B	108	MFAP4	180	OSCAR	252	PPP2R1B	324	RPL22	396	Somatostatin	468	UNC45A
37	Kilon	109	MFI2	181	OSM R beta	253	PPP2R4	325	RPL5	397	SORD	469	UNC5H4
38	KLK-B1	110	mGLUR5	182	Osteoadherin	254	PRCP	326	RPL7A	398	SorLA	470	UPB1
39	KMD4B	111	MGP	183	OXT	255	PRDM13	327	RPLP0	399	SOX4	471	UQCRB
40	KMT2B	112	Mimecan	184	p16 ARC	256	PRDX 1	328	RPS10	400	SP-D	472	UQCRH
41	KRT31	113	MINPP1	185	P20Sb3	257	PRELP	329	RPS11	401	Spectrin beta-5	473	URB
42	KRT72	114	MLCK	186	p23	258	PREP	330	RPS12	402	SPEN	474	URB2
43	Krt73	115	MMR	187	p39	259	PRG2	331	RPS19	403	SPINK7	475	UROC1
44	KRT82	116	MMRN1	188	P4HB	260	PRNP	332	RPS2	404	SPTBN1	476	UROD
45	KRT85	117	MN1	189	p73	261	Profilin 1	333	RPS20	405	Src	477	URP2
46	KRTDAP	118	Moesin	190	PA2G4	262	Properdin	334	RPS23	406	SREC-II	478	USP14
47	KRTHA3B	119	MP1	191	PABP	263	Prosaposin	335	RPS25	407	STAT3	479	USP5
48	KSR1	120	MPCA	192	PACS1	264	PTGDS	336	RPS28	408	Stathmin 1	480	Uteroglobin
49	LAD	121	MPO	193	PARVB	265	PSMB6	337	RPS3	409	SCP2	481	Utrophin
50	LAF4	122	MRP 1	194	PCBP1	266	PSMA3	338	RPS5	410	STI1	482	VAP-1
51	LAIR1	123	MSH6	195	PCBP2	267	PSMA5	339	RREB1	411	STOM	483	VAP-A
52	LAM b1	124	mTOR	196	PCCA	268	PSMB7	340	RSU1	412	SUCLG1	484	VCP
53	LAMA	125	MUCDHL	197	PCDH7	269	PSMD5	341	S100A1	413	SUMO3	485	VDAC1
54	LMNA	126	Multimerin 2	198	PCDX8	270	PSMB1	342	S100A11	414	Symplekin	486	Versican
55	LMNB1	127	MyBPC3	199	PCK2	271	PSMA6	343	S100A7	415	SynCAM	487	Vimentin B
56	LMNB2	128	MYH2	200	PCMT1	272	PSB2	344	S100A9	416	Syntaxin 7	488	VNN1
57	LAMA2	129	MYH6	201	PCNA	273	PSB4	345	S100P	417	TAB182	489	VSIG4
58	LAMB2	130	MYH7	202	PCPE-1	274	Protein C	346	TIM-4	418	TAGLN2	490	WDR1
59	LAMC1	131	MYHC	203	PCSK9	275	Protein Z	347	SAA4a	419	Talin1	491	WISP2
60	LAMP	132	MYL12B	204	PCYOX1	276	Prouroguanylin	348	aAmylase	420	Talin1&2	492	WNK2
61	LAMP1	133	MYL3	205	PDE1B	277	PRSS23	349	SAMSN1	421	TAX1BP3	493	YB1
62	LAMP2	134	MYO5A	206	PDIA6	278	PRSS3	350	SBP-1	422	TBCA	494	YY1
63	LAP3	135	Myoferlin	207	PDLIM1	279	PRTN3	351	SBSN	423	TCEB2	495	ZBTB4
64	LASP1	136	Myosin 18B	208	PDLIM5	280	PSMA1	352	SDF4	424	Tcf20	496	ZC3H4-N-t
65	LTBP2	137	Myotrophin	209	PDZD2	281	PSMA2	353	SDNSF	425	TCN1	497	ZC3H8
66	LCAT	138	NABC1	210	PEBP4	282	PSMA4	354	SDPR	426	TCP1 eta	498	ZDHHC18
67	LCMT2	139	NAGLU	211	PEPD	283	PSMA7	355	SCG5	427	Tenascin C	499	ZNF671
68	LDHA	140	NAP1L1	212	PER1	284	PSMB5	356	Semaphorin 6B	428	Tenascin X	500	Zyxin
69	LDHB	141	NAPRT1	213	perilipin 3	285	PSMC3	357	Semaphorin 7A	429	TFF2		
70	LEDGF	142	NASP	214	Perilipin-1	286	PSMD1	358	SEMG1	430	TGM3		
71	SPINK5	143	NCAM2	215	Periostin	287	PSMD9	359	SEMG2	431	Thioredoxin-1		
72	LILRA3	144	Nebulin	216	Peroxiredoxin 2	288	PTEN	360	Serpin A11	432	THOP1		

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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