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

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

User Manual

(Revised Mar. 20th, 2024)

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

RayBiotech
Empowering your proteomics

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RayBiotech Life, Inc.

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

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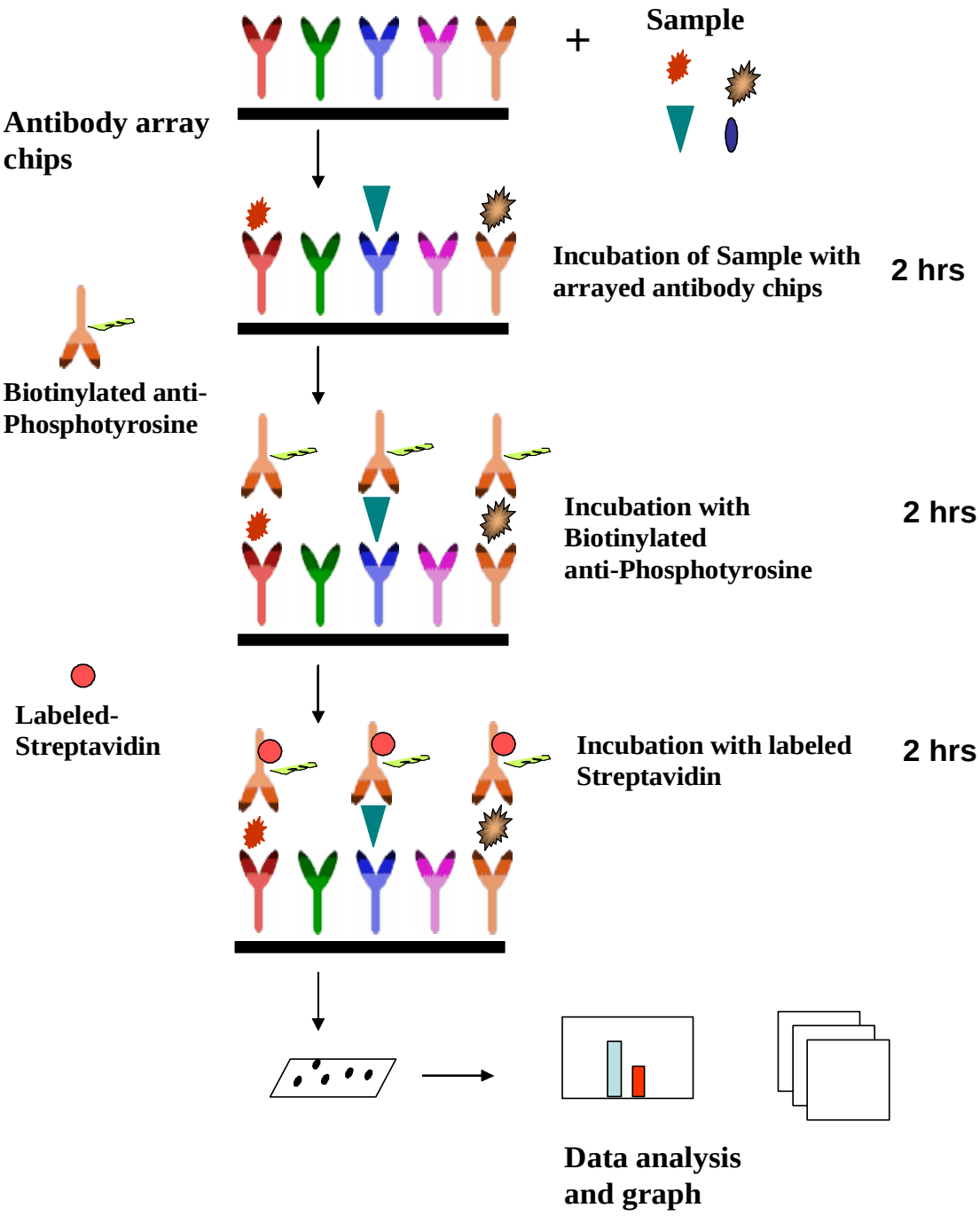
I. Introduction

Protein phosphorylation plays an unusually prominent role in cell signaling, development and growth. The RayBio® G-Series Mouse 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® Mouse Protein Tyrosine Phosphorylation Antibody Array 2 is specifically designed for simultaneous identification of the relative levels of phosphorylation of 500 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 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	AAM-PTYR-G2-4	AAM-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				

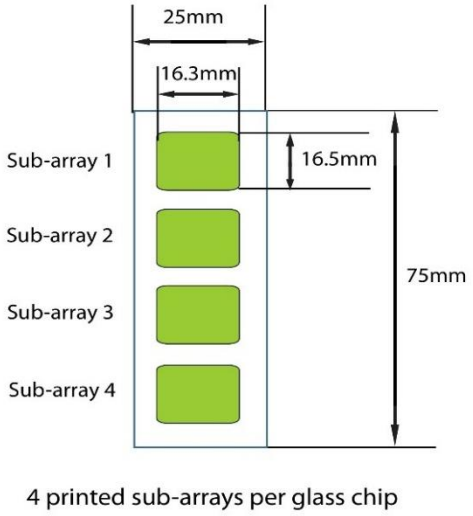
*Each slide contains 4 identical subarrays

**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.

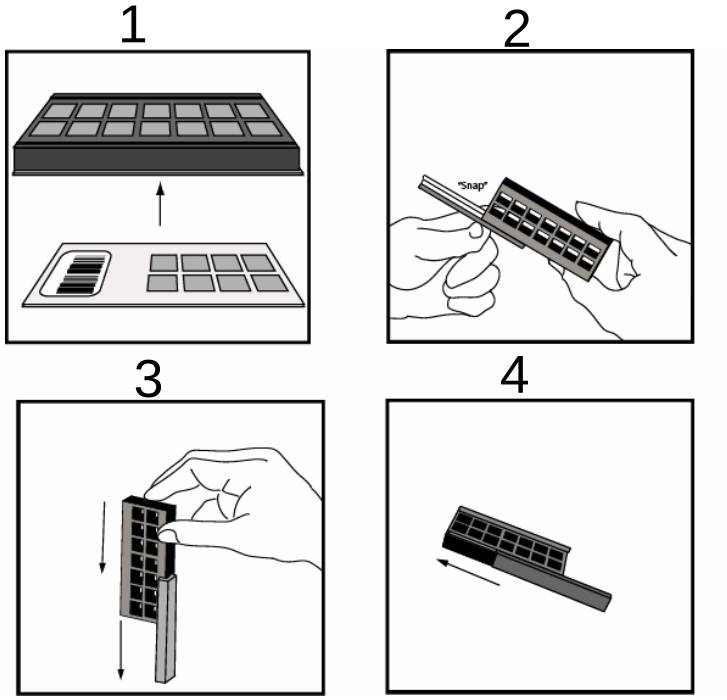
9. Remove all of Wash Buffer II from each well. Add 400 µl of the 1X Fluorescent dye-Conjugated Streptavidin solution to each subarray. Cover the incubation chamber with the Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in a dark room.

   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 Mouse 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 Mouse 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
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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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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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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
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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 Mouse Protein Tyrosine Phosphorylation Antibody Array 2 Target List

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	ABI3BP	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	B4GalT1	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	Gastrokine 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	IGF2BP2	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	MFI2
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	Advillin	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	C1QA	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	ITIHC4	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	GOLIM4	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	KIAA0319L	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	Neurotrimin
62	ANP32A	134	Cathepsin Z	206	Cystatin	278	FDPS	350	Histone H2A	422	LDH-H	494	NF-M
63	Antithrombin III	135	CBS	207	CYTL1	279	FGG	351	Histone H2A.Z	423	LEDGF	495	NIF3L1
64	APLP1	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	LIMS1	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	ARTS1	144	CD157	216	Cytokeratin 9	288	FKBP51	360	hnRNP A1	432	LTBP4		

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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