RayBio® G-Series Human Pathway Explorer Phosphorylation Antibody Array 1

For Simultaneously Detecting the Relative Levels of Phosphorylation of 393 Human Signaling Pathway Proteins

User Manual
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Cat#: AAH-PEX-G1-4 (4 Sample Kit)
Cat#: AAH-PEX-G1-8 (8 Sample Kit)

RayBiotech
Empowering your proteomics

ISO 13485:2016

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RayBiotech Inc.

RayBio® G-Series Human Pathway Explorer Phosphorylation Antibody Array 1 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 Pathway Explorer Phosphorylation Antibody Array 1 is a very rapid, convenient, and sensitive assay that can simultaneously detect multiple protein phosphorylation events and be used to monitor the activation or function of important biological pathways.

RayBiotech is committed to developing a comprehensive series of phosphorylation antibody arrays to advance your research. RayBio Human Pathway Explorer Phosphorylation Antibody Array 1 is specifically designed for simultaneous identification of the relative levels of phosphorylation of 393 different human signaling pathway proteins in cell lysate. By monitoring the changes in protein 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 Pathway Explorer Phosphorylation Antibody Array 1, treated or untreated cell lysate is added into antibody array glass slide wells. The antibody array slide wells are washed, and biotinylated anti-phospho Tyr/Thr/Ser antibodies are then used to detect the phosphorylated Tyrosine/Threonine/Serine 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.

II. MATERIAL PROVIDED

Store kit at ≤ -20 °C immediately upon arrival. Kit must be used within the 6-month expiration date.

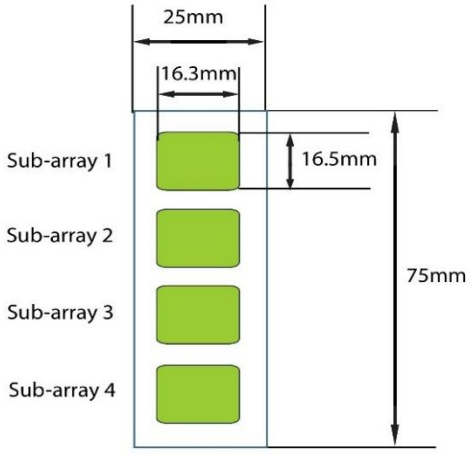
<table>
<tr><th>COMPONENT</th><th>AAH-PEX-G1-4</th><th>AAH-PEX-G1-8</th><th>STORAGE TEMPERATURE AFTER THAWING**</th></tr>
<tr><td>Array Glass Slide*</td><td>1</td><td>2</td><td rowspan="2">≤ -20°C</td></tr>
<tr><td>Blocking Buffer</td><td>1 bottle (8ml/ea)</td><td>2 bottles (8ml/ea)</td></tr>
<tr><td>Biotinylated Anti-Phospho Tyr/Thr/Ser Antibodies</td><td>1 vial</td><td>2 vials</td><td>2-8°C</td></tr>
<tr><td>Fluorescent Dye-Conjugated Streptavidin (Cy3 equivalent)</td><td>1 vial</td><td>2 vials</td><td>2-8°C</td></tr>
<tr><td>20X Wash Buffer I Concentrate</td><td colspan="2">1 bottle (30 ml)</td><td rowspan="3">2-8°C</td></tr>
<tr><td>20X Wash Buffer II Concentrate</td><td colspan="2">1 bottle (30 ml)</td></tr>
<tr><td>Wash Buffer III</td><td colspan="2">1 bottle (20 ml)</td></tr>
<tr><td>2X Cell Lysis Buffer Concentrate</td><td colspan="2">1 bottle (10ml)</td><td>2-8°C</td></tr>
<tr><td>Protease Inhibitor Cocktail</td><td colspan="2">1 vial</td><td rowspan="3">≤ -20°C</td></tr>
<tr><td>100x Phosphatase Inhibitor Cocktail I</td><td colspan="2">1 vial</td></tr>
<tr><td>Phosphatase Inhibitor Cocktail II</td><td colspan="2">1 vial</td></tr>
<tr><td colspan="4">Adhesive film</td></tr>
</table>

*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 gently 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, 20 µl 100x Phosphatase Inhibitor Cocktail Set I 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-Phospho Tyr/Thr/Ser Antibodies:** Briefly spin down the Detection Antibody vial before use. Add 90 µl of Blocking Buffer to the vial to prepare a Biotinylated Anti-phospho Tyr/Thr/Ser 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-phospho Tyr/Thr/Ser 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.

7. **Wash Buffer III and Blocking Buffer:** ready to use

V. OVERVIEW AND GENERAL CONSIDERATIONS

A. Preparation of Cell Lysates

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 array slide, 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 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 powder-free gloves.
- Dry the glass slide completely before the addition of the Blocking Buffer.
- Avoid breaking the glass slide when removing the chamber assembly.
- Handle the glass slide in a clean environment.

C. Incubation

- Completely cover the array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- To avoid evaporation, seal the incubation chamber with the provided 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-phospho Tyr/Thr/Ser 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 (page 10) onwards.

VI. PROTOCOL

A. Dry the Glass Slide

Open the pouch 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 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: To aspirate liquid samples or reagents from wells, gently place the pipette tip only in the corners of the well. Do not scrape the pipette tip across the surface of the slide.

   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.

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 (ensure the slide is completely submerged), and wash at room temperature with gentle shaking for 20 min.

5. Decant the Wash Buffer I from each well. Put the glass slide into a box with Wash Buffer II (ensure the slide is completely submerged), 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-phospho Tyr/Thr/Ser 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.

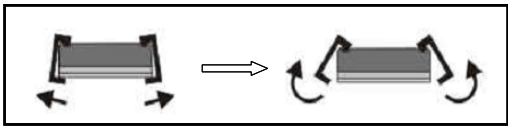
9. Remove all of Wash Buffer II from each well. Add 400 µl of the 1X Fluorescent Dye-Conjugated Streptavidin solution to each subarray. Cover the incubation chamber with the adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in a dark room.

   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 solution and disassemble the glass slide from the incubation frame and chamber. Disassemble the device by pushing the clips outward from the side, as shown below. Carefully separate the glass slide from the gasket, taking care not to touch the printed surface of the slide.



12. Gently place the glass slide into a 50 ml centrifuge tube or a plastic box with 40 ml of 1X Wash Buffer I as illustrated below. Wash with gentle rocking or shaking 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. Remove the water droplets from the slide surface by applying suction gently with a pipette tip. Place the glass slide in a laminar flow hood for 20 minutes or until the slide is completely dry. Place the slide under an aluminum foil tent to protect it from light. Make sure the slides are absolutely dry before scanning or storage.

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 Pathway Explorer 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 sites may not be recognized by biotinylated anti-phospho Tyr/Thr/Ser because of steric hindrance of the recognition site.

RayBio G-Series Human Pathway Explorer 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	29	30
1	POS1	POS1	POS2	POS2	Neg	Neg	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12
2	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	27	27
3	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	42	42
4	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
5	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	72	72
6	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	87	87
7	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	102	102
8	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	117	117
9	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	132	132
10	133	133	134	134	135	135	136	136	137	137	138	138	139	139	140	140	141	141	142	142	143	143	144	144	145	145	146	146	147	147
11	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	162	162
12	163	163	164	164	165	165	166	166	167	167	168	168	169	169	170	170	171	171	172	172	173	173	174	174	175	175	176	176	177	177
13	178	178	179	179	180	180	181	181	182	182	183	183	184	184	185	185	186	186	187	187	188	188	189	189	190	190	191	191	192	192
14	193	193	194	194	195	195	196	196	197	197	198	198	199	199	200	200	201	201	202	202	203	203	204	204	205	205	206	206	207	207
15	208	208	209	209	210	210	211	211	212	212	213	213	214	214	215	215	216	216	217	217	218	218	219	219	220	220	221	221	222	222
16	223	223	224	224	225	225	226	226	227	227	228	228	229	229	230	230	231	231	232	232	233	233	234	234	235	235	236	236	237	237
17	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	252	252
18	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	267	267
19	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	282	282
20	283	283	284	284	285	285	286	286	287	287	288	288	289	289	290	290	291	291	292	292	293	293	294	294	295	295	296	296	297	297
21	298	298	299	299	300	300	301	301	302	302	303	303	304	304	305	305	306	306	307	307	308	308	309	309	310	310	311	311	312	312
22	313	313	314	314	315	315	316	316	317	317	318	318	319	319	320	320	321	321	322	322	323	323	324	324	325	325	326	326	327	327
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24	343	343	344	344	345	345	346	346	347	347	348	348	349	349	350	350	351	351	352	352	353	353	354	354	355	355	356	356	357	357
25	358	358	359	359	360	360	361	361	362	362	363	363	364	364	365	365	366	366	367	367	368	368	369	369	370	370	371	371	372	372
26	373	373	374	374	375	375	376	376	377	377	378	378	379	379	380	380	381	381	382	382	383	383	384	384	385	385	386	386	387	387
27	388	388	389	389	390	390	391	391	392	392	393	393	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	POS2	POS2	POS1	POS1

RayBio G-Series Human Pathway Explorer Phosphorylation Antibody Array 1 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	14-3-3 zeta	67	CD61	133	ERa	199	IKKa	265	NFkb2	331	RYR2
2	14-3-3b	68	CDC25A	134	ErbB2	200	IKKb	266	NGFR	332	SCF R
3	4E-BP1	69	CDC25B	135	ErbB3	201	IL-10 R alpha	267	NR3C3	333	SHC
4	AB 1-40	70	CDC25C	136	ErbB4	202	IL-13 Ra1	268	P130Cas	334	SHIP1
5	ABL1	71	CDC37	137	ERK1/2	203	IL-2 sRa	269	P21	335	SHP-1
6	ACETYL CoA	72	CDK1	138	ERK2	204	IL-3 R alpha	270	P27	336	SHP-2
7	ACK1	73	CDK2	139	Ezrin	205	IL-4 R	271	P300	337	Siglec-2
8	ADD1	74	CDK5	140	Factor 3	206	IL-7 R alpha	272	P38	338	SLP-76/LCP2
9	AKT	75	CDK7	141	FADD	207	iNOS	273	P53	339	Smad1
10	AKT1S1	76	c-Fos	142	FAK	208	Insulin R	274	P63	340	Smad2
11	ALK	77	CHK1	143	FAS	209	IntegrinB1	275	P70S6K	341	Smad2/3
12	AMPK b1	78	CHK2	144	FER	210	IRF3	276	PAK1	342	smad4
13	AMPK a1	79	c-Jun	145	FGFR1	211	IRS1	277	PAK2	343	Smad5
14	AR	80	Claudin-3	146	FGFR2	212	ITGB4	278	PAK3	344	SMC1
15	ARAF	81	CLDN6	147	FGFR2 (a isoform)	213	ITK	279	PAK4	345	SNCA
16	ARHGEF2	82	CLDN7	148	FGR	214	JAK1	280	PARP	346	SP1
17	ASK1	83	c-Myc	149	Filamin A	215	JAK2	281	Paxillin	347	SRC
18	ATF1	84	Cofilin	150	Flt-3	216	JAK3	282	PDGF Ra	348	SREBF1
19	ATF2	85	Cortactin	151	FosB	217	JNK	283	PDGF Rb	349	SRMS
20	ATF4	86	CPI17 alpha	152	FoxO1	218	JunB	284	PDK1	350	STAT1
21	ATM	87	CREB1	153	Foxo3	219	JunD	285	PEA15	351	STAT2
22	ATRIP	88	c-Rel	154	FRK	220	KCNA3	286	PECAM-1	352	STAT3
23	ATR	89	CRK	155	FRS2	221	KCNB1	287	PIM1	353	STAT4
24	AURKA	90	CRKL	156	FYN	222	KCNIP3	288	PIP5K3	354	STAT5
25	AURKB	91	CTNND1	157	G3BP	223	KSR1	289	PKC a	355	STAT5b
26	AURKC	92	CXCR4	158	GAB2	224	LaminA+C	290	PKC d	356	Stat6
27	AXL	93	Cyclin B1	159	GABRB1	225	LAT	291	PKC epsilon	357	STMN1
28	BAD	94	Cyclin D1	160	GAP43	226	LCK	292	PKC theta	358	Survivin
29	BAX	95	Cyclin E1	161	GATA-1	227	LIMK1	293	PKC zeta	359	SYK
30	BCL-2	96	Cyclin D2	162	GJA1	228	LKB1	294	PKD1	360	SYN1
31	BCL-XL	97	Cytokeratin 18	163	GRIA1	229	LSD1	295	PKD2	361	SYT1
32	BCR	98	Cytokeratin 8	164	GRIA2	230	LTK	296	PKMYT1	362	TAK1
33	Beclin1	99	DAB1	165	GRIN1	231	Lyn	297	PKR	363	Tau
34	beta-Arrestin 1	100	DAPP1	166	GRIN2A	232	MAP3K1	298	PLA2G4A	364	Tec
35	beta-Catenin	101	DAXX	167	Grin2b	233	MAP3K8	299	PLCB3	365	TGF-B2
36	BID	102	DDR1	168	GRK1	234	MAPK6	300	PLCg1	366	TGF-beta RI
37	BIM	103	DDX5	169	GRK2	235	MARCKS	301	PLCG2	367	Tie-1
38	BLK	104	DNM1	170	GSK3a	236	MATK	302	PLD1	368	Tie-2
39	BLNK	105	DOK1	171	GSk3b	237	M-CSF R	303	PLD2	369	TLK1
40	BMX	106	DTK	172	HCK	238	MDM2	304	PLK1	370	TNK1
41	B-raf	107	DUSP1	173	HDAC1	239	MDM4	305	PP2A	371	TOP2A
42	BRCA1	108	E2F1	174	HDAC2	240	MEF2A	306	PPARg2	372	TrkA
43	BTK	109	EEF-2	175	HDAC3	241	MEF2C	307	PPP1R1B	373	TrkB
44	CAMK1	110	EGFR	176	HDAC4	242	MEF2D	308	PRKACA	374	Tuberin / TSC2
45	CAMK2A	111	EIF2a	177	HDAC5	243	MEK1	309	PRKCB	375	TXK
46	CAMK2d	112	EIF4B	178	HDAC6	244	MEK2	310	PTEN	376	TYH
47	CAMK4	113	EIF4E	179	HDAC8	245	MET	311	PTK6	377	TYK2
48	Casein Kinase 1a	114	EIF4G1	180	His H2A.x	246	MITF	312	PYK2	378	TYRO10 (DDR2)
49	Casein Kinase 2b	115	ELK1	181	Histone H3	247	MKK3	313	Rac1	379	ULK1
50	Caspase 6	116	eNOS	182	HNF-4a	248	MKK4	314	RACGAP1	380	VASP
51	Caspase 7	117	EPB41	183	HRS	249	MKK6	315	RAD51	381	VAV1
52	Caspase 3	118	EphA1	184	HSF1	250	MKK7	316	Raf1	382	VAV2
53	Caspase 8	119	EphA2	185	HSL	251	MKNK1/MNK1	317	RASGRF1	383	VE-Cadherin
54	Caspase 1	120	EphA3	186	HSP27	252	MLKL	318	RB1	384	VEGF-R1
55	Caspase 2	121	EPHA4	187	HSP90	253	MSK1	319	RELB	385	VEGF-R2
56	Caspase 9	122	EphA5	188	ICAM-1	254	MSK2	320	Ret	386	VEGFR3
57	Catalase	123	EphA6	189	ICK	255	MSP/MST1	321	RGS16	387	Vinculin
58	Caveolin-1	124	EphA7	190	IFN-a/b R1	256	mTOR	322	RhoA	388	WASF1
59	CBL	125	EphA8	191	IFN-gamma R1	257	MUCI	323	Rictor	389	WASP
60	CD19	126	EphB1	192	IGF-1 R	258	MUSK	324	ROR1	390	WNK1
61	CD247	127	EphB2	193	IGFBP-3	259	MYPT1	325	ROR2	391	WWOX
62	CD28	128	EphB3	194	IGF-II R	260	Nbs1	326	ROS	392	XIAP
63	CD32	129	EphB4	195	IkBa	261	NF2	327	RPS6	393	ZAP70
64	CD4	130	EphB6	196	IkB beta	262	NFATC3	328	RPS6KA3		
65	CD45	131	Ephrin B2	197	IkB epsilon	263	NFATC4	329	RSK1		
66	CD5	132	EPO R	198	IKK gamma	264	NF-KB (P65)	330	RYK		

VIII. TROUBLESHOOTING GUIDE

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	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. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Avoid bubble formation during incubation
	Arrays are not completed covered by reagent	Completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
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

IX. REFERENCE LIST

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