RayBio® G-Series Mouse Protein Tyrosine Phosphorylation Antibody Array 3

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

User Manual

(Revised Mar. 20th, 2024)

Cat#: AAM-PTYR-G3-4 (4 Sample Kit)
Cat#: AAM-PTYR-G3-8 (8 Sample Kit)

RayBiotech
Empowering your proteomics

We Provide You with Quality Products and Excellent Service

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

RayBiotech Life, Inc.

RayBio® G-Series Mouse Protein Tyrosine Phosphorylation Antibody Array 3 Protocol

TABLE OF CONTENTS

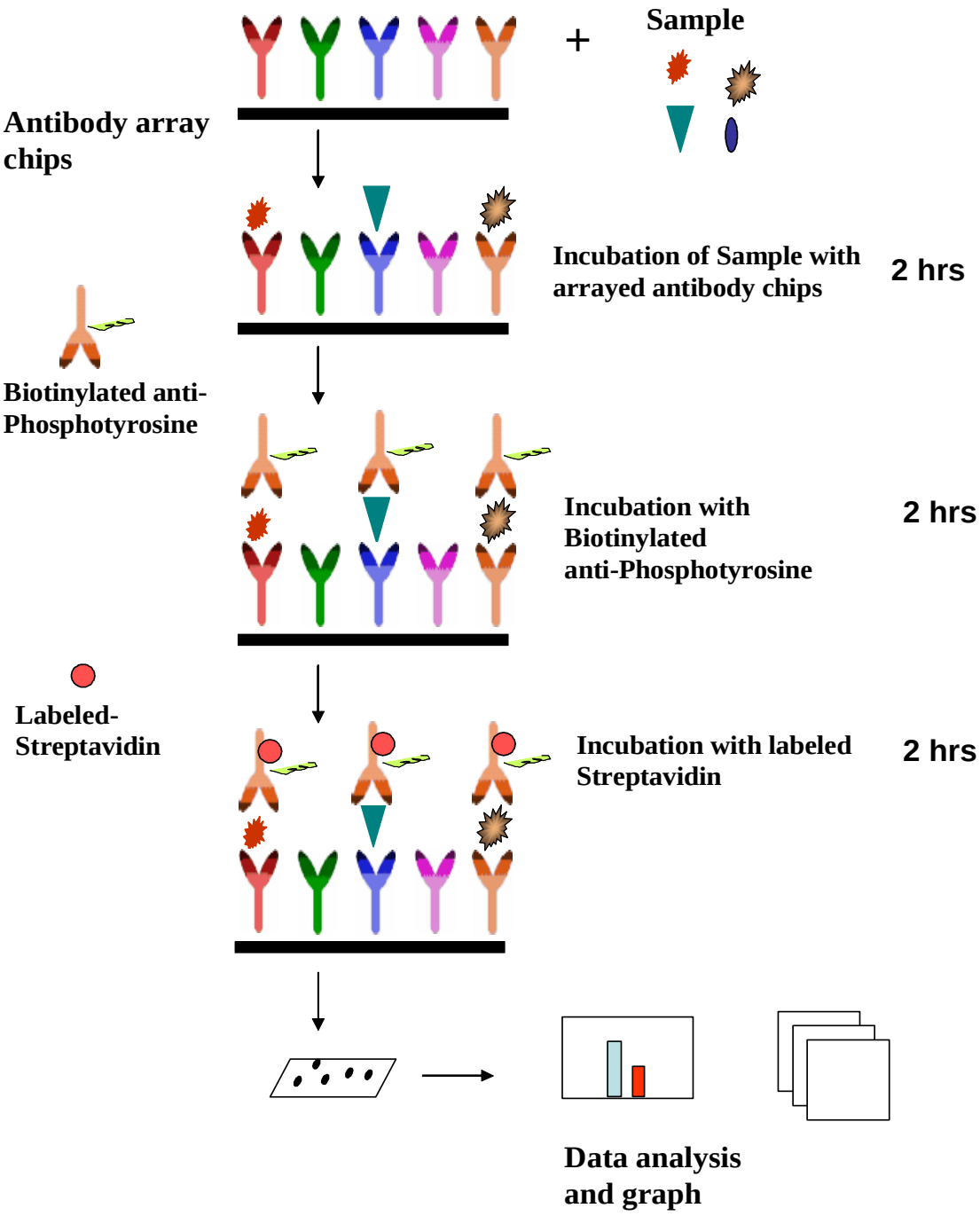
I. Introduction

Protein phosphorylation plays an unusually prominent role in cell signaling, development and growth. The RayBio® G-Series Mouse Protein Tyrosine Phosphorylation Antibody Array 3 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 3 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 3, 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-G3-4	AAM-PTYR-G3-8	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				

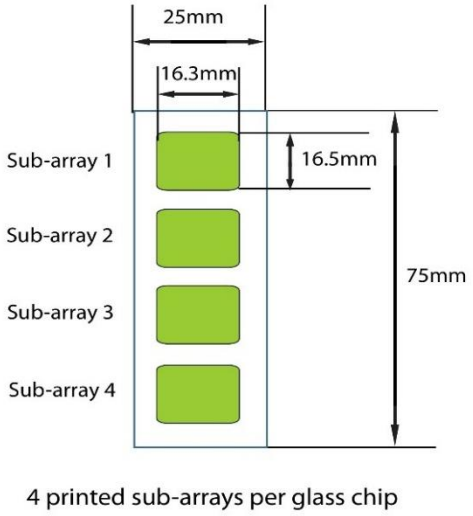
*Each slide contains 4 identical subarrays

**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.

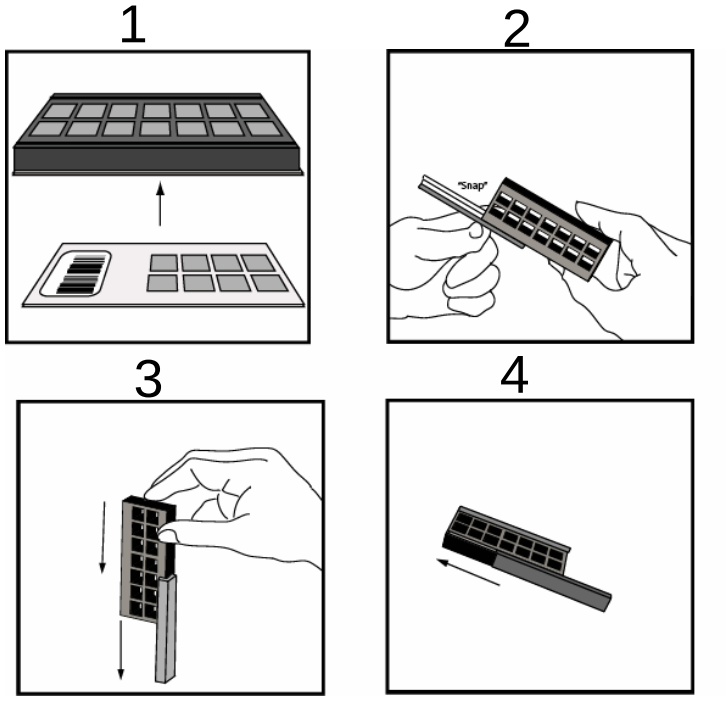
9. Remove all of Wash Buffer II from each well. Add 400 µl of the 1X Fluorescent dye-Conjugated Streptavidin solution to each subarray. Cover the incubation chamber with the Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in a dark room.

   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 3 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 3 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
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
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
10	132	132	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
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
12	162	162	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
13	177	177	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
14	192	192	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
15	207	207	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
16	222	222	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
17	237	237	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
18	252	252	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
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
22	308	308	309	309	310	310	311	311	312	312	313	313	314	314	315	315	316	316	317	317	318	318	319	319	320	320	321	321	322	322
23	323	323	324	324	325	325	326	326	327	327	328	328	329	329	330	330	331	331	332	332	333	333	334	334	335	335	336	336	337	337
24	338	338	339	339	340	340	341	341	342	342	343	343	344	344	345	345	346	346	347	347	348	348	349	349	350	350	351	351	352	352
25	353	353	354	354	355	355	356	356	357	357	358	358	359	359	360	360	361	361	362	362	363	363	364	364	365	365	366	366	367	367
26	368	368	369	369	370	370	371	371	372	372	373	373	374	374	375	375	376	376	377	377	378	378	379	379	380	380	381	381	382	382
27	383	383	384	384	385	385	386	386	387	387	388	388	389	389	390	390	391	391	392	392	393	393	394	394	395	395	396	396	397	397
28	398	398	399	399	400	400	401	401	402	402	403	403	404	404	405	405	406	406	407	407	408	408	409	409	410	410	411	411	412	412
29	413	413	414	414	415	415	416	416	417	417	418	418	419	419	420	420	421	421	422	422	423	423	424	424	425	425	426	426	427	427
30	428	428	429	429	430	430	431	431	432	432	433	433	434	434	435	435	436	436	437	437	438	438	439	439	440	440	441	441	442	442
31	443	443	444	444	445	445	446	446	447	447	448	448	449	449	450	450	451	451	452	452	453	453	454	454	455	455	456	456	457	457
32	458	458	459	459	460	460	461	461	462	462	463	463	464	464	465	465	466	466	467	467	468	468	469	469	470	470	471	471	472	472
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® G-Series Mouse Protein Tyrosine Phosphorylation Antibody Array 3 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	AARE	73	Filaggrin	145	PABP1	217	PREP	289	RPL22	361	SIM2	433	TRAP1
2	ACAT1	74	FITM1	146	PACS1	218	PRG2	290	RPL23A	362	SIRPB1	434	TRAP220
3	ACOT2	75	GARS	147	PNLIP	219	PrP	291	RPL3	363	Six3	435	TRF 2
4	ADAM28	76	GCC2	148	PARVB	220	Profilin 1	292	RPL32	364	SLC4A1	436	TRIM14
5	AHCY	77	GLI-2	149	PCAP	221	Prolargin	293	RPL4	365	SLITRK1	437	Tropomyosin 3
6	AK1	78	GLOD4	150	PCBP1	222	Prosaposin	294	RPL7	366	SLURP1	438	TRP-1
7	AKR1A1	79	GLUL	151	PCBP2	223	PTGDS	295	RPL7A	367	SMAD6	439	TRPS1
8	ALDH2	80	GMPR1	152	PCCA	224	PSMD2	296	RPLP0	368	SMC4	440	Trypsinogen-2
9	DEFA5	81	GOLGA3	153	PCDH12	225	Protein C	297	RPLP2	369	SMPD4	441	TSR2
10	ANKRD9	82	GP2	154	PCDH8	226	Protein Z	298	RPS10	370	SNRPD1	442	TTC3
11	ANXA3	83	gp340	155	PCK2	227	PRR4	299	RPS11	371	SOD1	443	TTF1
12	AP180	84	GTF2F1	156	PCMT1	228	PRRC2A	300	RPS12	372	SOD2	444	TUBA6
13	AP3S2	85	HA1	157	PCNA	229	PRSS23	301	RPS13	373	SOD-3	445	TWF2
14	APLP2	86	HARS	158	PCPE-1	230	PRSS3	302	RPS14	374	Somatoliberin	446	TXNDC15
15	ApoA V	87	HIC1	159	PCSK9	231	PRTN3	303	RPS15A	375	Somatostatin	447	TXNDC4
16	ASPM	88	HIP55	160	PDAP1	232	PSMA1	304	RPS16	376	SORD	448	TXNDC5
17	ASS1	89	H1F0	161	PDE1B	233	PSMA2	305	RPS18	377	SorLA	449	TXNRD2
18	ATOX1	90	HIST1H1B	162	PDIA6	234	PSMA4	306	RPS19	378	SOX4	450	UBA1
19	ATPG	91	HIVEP2	163	PDLIM1	235	PSMA5	307	RPS2	379	SOX5	451	UBE2D3
20	AUTS2	92	hnRNP K	164	PDLIM3	236	PSMA6	308	RPS20	380	SP-D	452	Ube2L3
21	BAI2	93	hnRNP R	165	PDZD2	237	PSMB1	309	RPS23	381	Spectrin	453	UBE2N
22	BarX1	94	HNRNPUL2	166	PEBP1	238	PSMB2	310	RPS25	382	SPEN	454	UCH-L1
23	BBS1	95	HNRPA3	167	PEBP4	239	PSMB3	311	RPS3	383	SPG48	455	UFM 1
24	UBC9	96	HP1 gamma	168	PENK	240	PSMB4	312	RPS3A	384	SPINK5	456	UGGT
25	BLM	97	Importin 7	169	PEPD	241	PSMB5	313	RPS4X	385	SPS2L	457	CMPK1
26	BOLA2	98	Involucrin	170	perilipin-3	242	PSMB6	314	RPS5	386	SPTBN2	458	UNC13D
27	C10orf58	99	ISLR	171	Perilipin-1	243	PSMB7	315	RPS8	387	SPTLC1	459	UNC45A
28	CACNA1H	100	ITPR2	172	Periostin	244	PSMC3	316	RPS9	388	Src	460	UNC5H4
29	Calpain-2	101	ITPR3	173	Periplakin	245	PSMD1	317	RREB1	389	SSC5D	461	UPB1
30	CaMK2	102	KCNAB3	174	Peroxiredoxin-2	246	PSMD5	318	RSF1	390	STAT3	462	UQCRB
31	CaMK2D	103	LAMA5	175	Peroxiredoxin-3	247	PSMD9	319	RSU1	391	Stathmin 1	463	UQCRH
32	CBL	104	LDB3	176	Peroxiredoxin-1	248	PSME1	320	RUSC1	392	STI1	464	URB
33	CBR1	105	LHPP	177	PFAS	249	PSME2	321	Septin 7	393	STOM	465	URB2
34	CCDC58	106	LIPG	178	PFDN6	250	PTBP1	322	S100A1	394	STXBP2	466	UROC1
35	CCT6A	107	MAP4K4	179	PFKL	251	PTEN	323	S100A11	395	SUCLG1	467	UROD
36	CHCHD3	108	MICALL2	180	PGAM1	252	PTGR1	324	S100A7	396	SUMO3	468	Uroguanylin
37	Cingulin	109	MON2	181	PGAM2	253	PTK7	325	S100A9	397	SVEP1	469	URP2
38	CIT	110	MPST	182	PGK-1	254	PTMA	326	SDC4	398	Symplekin	470	USP14
39	CMG1	111	MRC2	183	PGLS	255	PTPRG	327	SAA4	399	SynCAM	471	USP2
40	CNBP	112	MSH3	184	PG-M	256	PTPRK	328	SBP-1	400	Synemin	472	USP5
41	CNPY2	113	MTA2	185	PGM1	257	PTPRM	329	SC35	401	SYNPO2L	473	Uteroglobin
42	Coilin	114	MTHFD1	186	PGRPL	258	PTPRZ	330	SCG	402	Syntaxin 7	474	Utrophin
43	COL8A2	115	MUC5B	187	PHGDH	259	PZP	331	SCN3A	403	TAB182	475	VARS
44	COLEC11	116	MVD	188	Piccolo	260	QARS	332	SCP2	404	Talin1	476	VAP-1
45	COPG2	117	Myosin IIB	189	pIgR	261	QDPR	333	SDNSF	405	TARS	477	VAP-A
46	CORO1B	118	NACA1	190	PIK3C2B	262	QPRT	334	SDPR	406	TAX1BP3	478	VCP
47	CPA3	119	NAGPA	191	PIN	263	Quiescin Q6	335	SECISBP2	407	TBCA	479	VDAC1
48	CPI17 alpha	120	NAV2	192	PIP5K2 alpha	264	Rab1A	336	Secretogranin V	408	TCEB2	480	VILIP3
49	CrkRS	121	NFATC4	193	PISD	265	Rab7a	337	Semaphorin 6B	409	Tcf20	481	Vimentin
50	CRLF3	122	NNT	194	PLA2G1B	266	Ran	338	Semaphorin 7A	410	TCP1 delta	482	VNN1
51	CSRP3	123	NPEPPS	195	Plastin 3	267	RanBP1	339	SERBP1	411	TCP1 eta	483	VPS4B
52	CTNNAL1	124	NQO2	196	Plastin L	268	RanGAP1	340	Serpin A11	412	TCP1 theta	484	VSIG4
53	CTNND1	125	NSFL1C	197	PLBD2	269	RAP1B	341	Serpin A7	413	TCTP	485	WDR1
54	Cyclophilin F	126	NUCB1	198	PLD4	270	Rbm15	342	Serpin B3D	414	TDIF2	486	WDR44
55	Cystatin C	127	NUP214	199	Plectin	271	RCL	343	Serpin B6	415	Tenascin C	487	WISP2
56	DCAMKL1	128	OAF	200	Plexin B1	272	RECQ4	344	Serpin B8	416	Tenascin XB	488	WNK2
57	Dematin	129	OIT3	201	Plexin B2	273	Reg3A	345	Serpin F2	417	TFF2	489	XPG
58	DIAPH1	130	OPCML	202	PLOD1	274	REV3L	346	Serpin H1	418	TGM3	490	YB1
59	DKC1	131	ORM2	203	PLOD2	275	RHOC	347	Serpin A10	419	Thioredoxin-1	491	SYN1
60	DLST	132	OSBP1	204	Plxdc2	276	RHOG	348	SERPINB1	420	THOP1	492	YY1
61	DMRT1	133	OSCAR	205	PMCA	277	RNASE1	349	SerpinB4	421	TIF1 alpha	493	ZAK
62	Dystrophin	134	OSM R beta	206	PNP	278	RNASET2A	350	SerpinE2	422	TMEM103	494	zbtb11
63	Ebf4	135	Osteoadherin	207	POLD2	279	RLF	351	SerRS	423	TOB2	495	ZBTB4
64	EBP50	136	OTC	208	POLR2A	280	RNASE4	352	SET	424	TOMM70A	496	ZC3H18
65	ECHDC1	137	OTUB1	209	POR	281	Rnose2	353	SEZ6L2	425	TOP2B	497	ZC3H4
66	EHHADH	138	OTUD7A	210	PPOX	282	RP1	354	SF20	426	TPD52L2	498	ZC3H8
67	EIF3D	139	OT-NPI	211	PPP1CC	283	RPL10	355	SHANK1	427	TPM4	499	ZNF295
68	eIF4A2	140	p16 ARC	212	PPP1R9A	284	RPL10A	356	SHC1	428	TPP1	500	Zyxin
69	eIF4GII	141	p23	213	PPP2R1B	285	RPL11	357	SHMT1	429	TPPP3		
70	ENDOD1	142	p39	214	PPP2R4	286	RPL12	358	SHOX	430	TPR		
71	EYA2	143	P4HB	215	PRCP	287	RPL14	359	SHP-1	431	TALDO1		
72	F8	144	p73	216	PRDM13	288	RPL17	360	Siglec-1	432	Transthyretin		

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

1. Profiling receptor tyrosine kinase activation by using Ab microarrays. Nielsen UB, Cardone MH, Sinskey AJ, MacBeath G, and Sorger PK. **PNAS.** 2003;100(16):9330-9335.

2. A Prototype Antibody Microarray Platform to Monitor Changes in Protein Tyrosine Phosphorylation. Gembitsky DS, Lawlor K, Jacovina A, Yaneva M, and Tempst P**. Mol Cell Proteomics.** 2004;3:1102–1118.

3. Analysis of receptor signaling pathways by mass spectrometry: Identification of Vav-2 as a substrate of the epidermal and platelet derived growth factor receptors. Pandey A, Podtelejnikov AV, Blagoev B, Bustelo XR, Mann M, and Lodish HF. **PNAS.** 2000; 97(1);179–184.

4. Reduced T-cell and dendritic cell function is related to cyclooxygenase-2 overexpression and protaglandin e(2) secretion in patients with breast cancer". Pockaj BA, Basu GD. **Annal Surg Oncol**. 2004;3:327-344.

5. Cytokine Antibody Arrays: A Promising Tool to Identify Molecular Targets for Drug Discovery. Huang RP. **Comb Chem High Throughput Screen**. 2003,;6:79-99.

6. Connexin suppresses human glioblastoma cell growth by down-regulation of monocyte chemotactic protein 1, as discovered using protein array technology. Huang R, Lin Y, Wang CC, J et al. **Cancer Res**. 2002;62:2806-2812.

7. Profiling of cytokine expression by biotin-labeled-based protein arrays. Lin Y, Huang R, Chen L-P, et al. **Proteomics**. 2003, 3: 1750-1757.

8. A novel method for high- throughput protein profiling from conditioned media and patient's sera. Huang RP, Huang R, Fan Y, and Lin Y. **Ana. Biochem.** 2001;294(1):55-62.

RayBio® Cytokine Antibody Arrays are patent-pending technology developed by RayBiotech.

This product is intended *for research only* and is not to be used for clinical diagnosis. Our produces may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech Life, Inc.

Under no circumstances shall RayBiotech be liable for any damages arising out of the use of the materials.

Products are guaranteed for 6 months from the date of purchase when handled and stored properly. In the event of any defect in quality or merchantability, RayBiotech's liability to buyer for any claim relating to products shall be limited to replacement or refund of the purchase price.

RayBio® is a registered trademark of RayBiotech Life, Inc.

This product is for research use only.