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

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

User Manual

(Revised Mar. 20th, 2024)

Cat#: AAH-PTYR-G3-4 (4 Sample Kit)
Cat#: AAH-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 Human Protein Tyrosine Phosphorylation Antibody Array 3 Protocol

TABLE OF CONTENTS

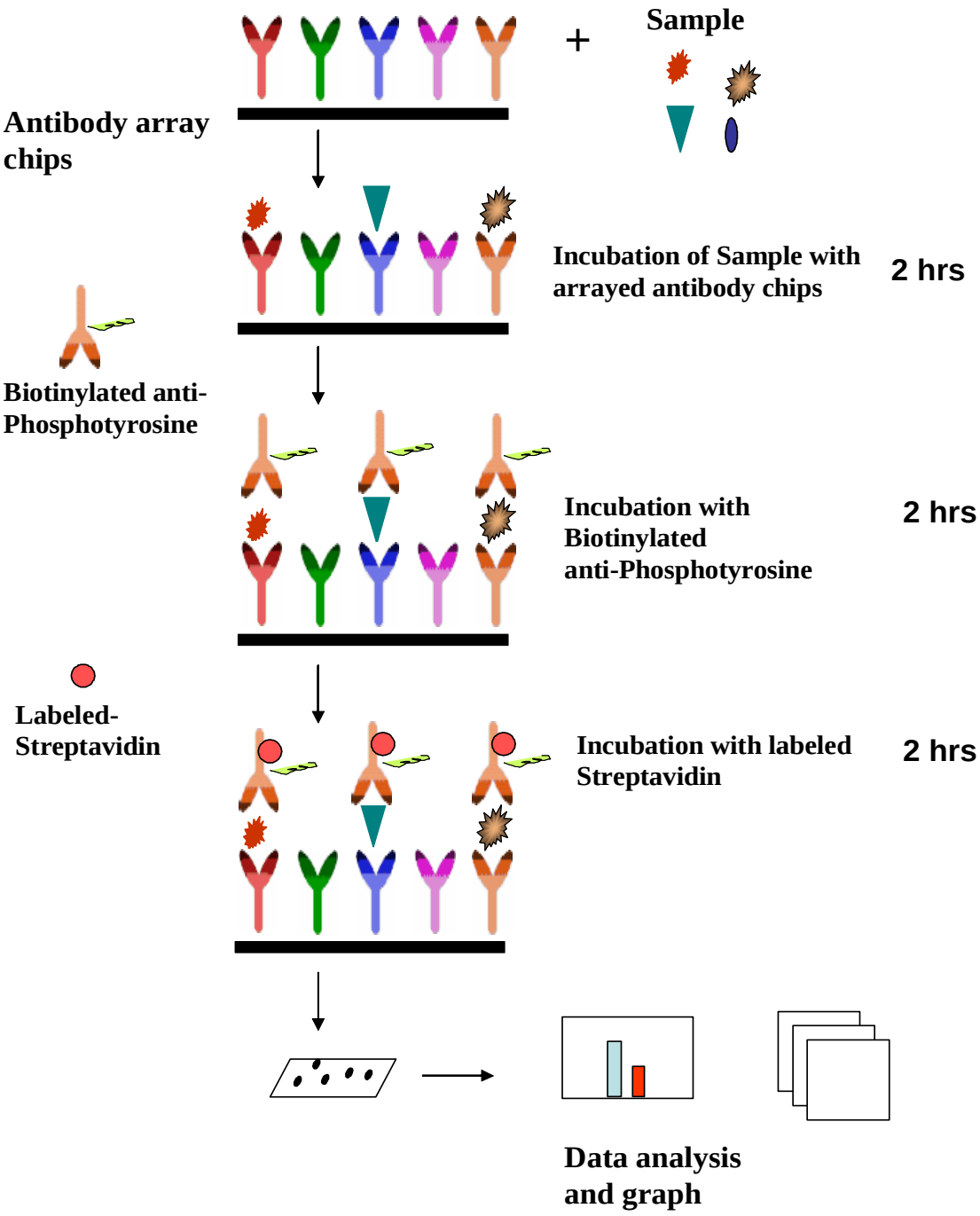
I. Introduction

Protein phosphorylation plays an unusually prominent role in cell signaling, development and growth. The RayBio® G-Series Human 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® Human Protein Tyrosine Phosphorylation Antibody Array 3 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 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

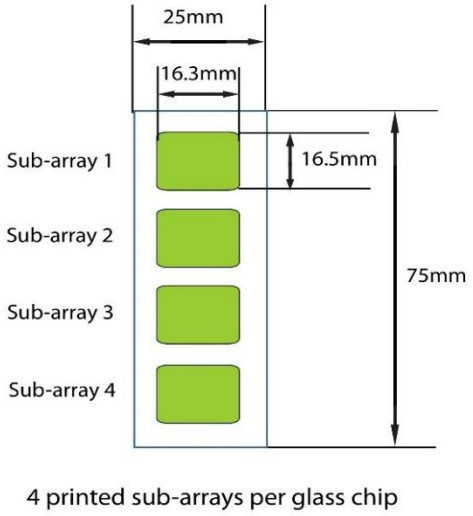
Upon receipt, the kit should be stored at -20 °C to -80 °C. Please use within 6 months from the date of shipment. After initial use, the 2X Cell Lysis Buffer, Blocking Buffer, 20X Wash Buffer I, 20X Wash Buffer II, Biotin-Conjugated Anti-phosphotyrosine and Fluorescent dye-Conjugated Streptavidin (Cy3 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 3 Glass Slide with Frame (each slide contains 4 Subarrays, with 1 slide included for AAH-PTYR-G3-4 (4 Sample Kit), and 2 slides included for AAH-PTYR-G3-8 (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.

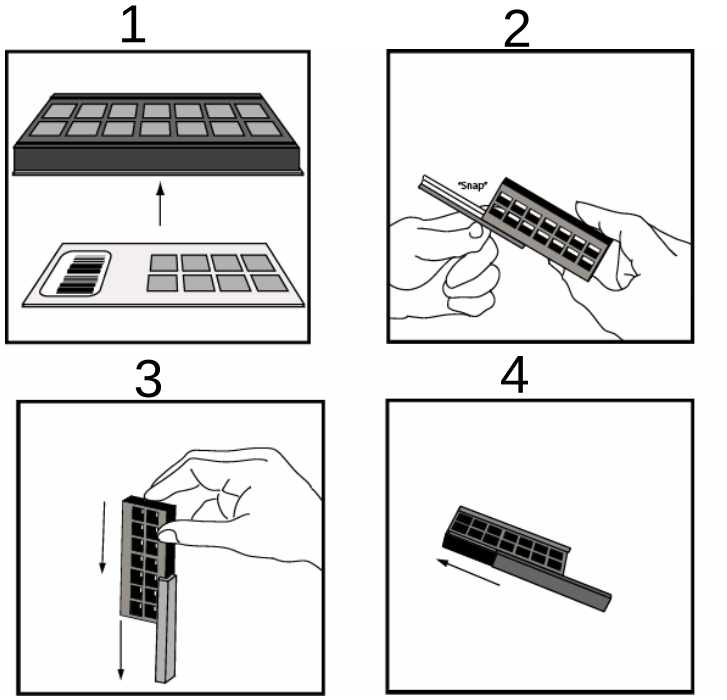
9. Remove all of Wash Buffer II from each well. Add 400 µl of the 1X Fluorescent dye-Conjugated Streptavidin solution to each subarray. Cover the incubation chamber with the Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in a dark room.

   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 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 Human 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 Human Protein Tyrosine Phosphorylation Antibody Array 3 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	14-3-3 beta	73	Antithrombin III	145	C4BPA	217	CHREBP	289	Cytokeratin 9	361	EVC2	433	Glyoxalase II
2	14-3-3 epsilon	74	APA	146	C5b-9	218	Chromogranin B	290	D4 GDI	362	Ezrin	434	GM2A
3	14-3-3 eta	75	APLP-1	147	C6	219	Chromogranin C	291	DAK	363	F11	435	GMF beta
4	14-3-3 gamma	76	APM2	148	C8G	220	CIP29	292	Contactin-4	364	FABP5	436	GNB1
5	14-3-3 sigma	77	Apo (a)	149	C9orf40	221	CKB	293	DARS2	365	Factor IX	437	GNPTG
6	14-3-3 theta	78	APOA1BP	150	CA1	222	CLIC1	294	DCI	366	Factor V	438	GOLPH2
7	14-3-3 zeta	79	ApoF	151	CA150	223	CLIC4	295	DCXR	367	Factor XII	439	GOLPH4
8	53BP1	80	ApoL1	152	CA2	224	CLIP170	296	DDAH1	368	Factor XIII	440	GOT2
9	67LR	81	ApoL2	153	CA3	225	CL-P1	297	DDT	369	FAM20C	441	GPR116
10	ABAT	82	ARFBP1	154	CACNB4	226	CLPS	298	DDX3Y	370	FAM3C	442	GPLD1
11	ABCF1	83	ARFGEF3	155	CAD	227	CLTA	299	DEFA6	371	Fascin	443	GRHL1
12	ABI3BP	84	ASL	156	Cadherin 22	228	CNN2	300	DEP-1	372	FASN	444	Granzyme M
13	ACAA1	85	ArgRS	157	Cadherin-6	229	CNOT1	301	DNER	373	fast skeletal Myosin	445	GRHPR
14	ACAA2	86	ARP19	158	CALD1	230	CO4A2	302	Dermcidin	374	FASTKD5	446	GRP
15	ACACA	87	Arp2	159	CALML5	231	COG4	303	Desmocollin 1	375	FBP38	447	GSTM1
16	ACAA	88	ARP2/3	160	Calmodulin	232	COL19A1	304	Desmocollin-2	376	FBP2	448	GSTP1
17	ACLP	89	Arp3	161	Calpain 1	233	COL4A3	305	Desmocollin-3	377	FBPase 1	449	Guanylin
18	ACLY	90	ARPC2	162	Calpain S1	234	Col6A2	306	Desmoglein-1	378	FCGBP	450	GULP1
19	Aconitase 1	91	ARPC3	163	Calpastatin	235	COL9A3	307	Desmoglein-2	379	FDPS	451	H6PD
20	ACTBL2	92	ART3	164	Calretinin	236	COLEC10	308	Desmoplakin	380	FH	452	HABP2
21	ACTC1	93	ARTS1	165	Calumenin	237	Collagen I a1	309	Desmuslin	381	Fibrillin 1	453	HBZ
22	Actinin alpha 1	94	ARX	166	CAP1	238	Collagen III	310	Destrin	382	FGG	454	HCFC1
23	ADAMDEC1	95	ASH2L	167	CapG	239	Collagen IVa6	311	DGK	383	Fibrinogen-like 2	455	HDGF
24	ADAS	96	ASGR2	168	CAPZA1	240	Collagen IX	312	DISC 1	384	Fibrinopeptide B	456	HEG1
25	ADH1B	97	ASK1	169	CPB2	241	Collagen V	313	DMGDH	385	Fibulin 3	457	Hemoglobin
26	ADH1C	98	AST	170	CARHSP1	242	Collagen VI	314	DMRN9	386	Ficolin-2	458	Hemoglobin A1c
27	ADH4	99	DNPEP	171	Caspase-14	243	Collagen X	315	DBH	387	Filamin A	459	HBB
28	ADH5	100	ASXL1	172	Catalase	244	COL15A1	316	DOT1L	388	Filamin B	460	HBD
29	ADM	101	ATBF1	173	Cathelicidin	245	COMP	317	DPEP2	389	Filamin C	461	HBG2
30	Advillin	102	ATP5A	174	Cathepsin A	246	CFB	318	DPP3	390	FKBP12	462	HEXB
31	AFG3L2	103	ATP5O	175	Cathepsin G	247	Contactin-3	319	DPPI	391	FKBP25	463	HGFA
32	AGA	104	ATPB	176	Cathepsin H	248	COPS8	320	DRIL1	392	FKBP51	464	hGH
33	Aggrecan	105	B3GNT2	177	Cathepsin Z	249	Corneodesmosin	321	DSCAM	393	FLG2	465	hHR23b
34	AGXT	106	B4GalT1	178	CBS	250	Coronin 3	322	DSPG3	394	FOLR3	466	HIBADH
35	AHNAK	107	B7-H2	179	CCDC126	251	Cortactin	323	Dystroglycan	395	Frizzled 8	467	HINT1
36	Ahsp	108	B7-H3	180	CCDC25	252	COTL1	324	UBA1	396	FRY	468	HIP1R
37	AIF	109	BAD	181	CCT3	253	CPE	325	ECHS1	397	FSH	469	Histone H1.2
38	AK2	110	Band 3	182	CD109	254	CPEB3	326	ECM-1	398	Azurocidin	470	Histone H1.3
39	AKAP9	111	BASP1	183	CD133	255	CPM	327	EEF1G	399	FUCA1	471	Histone H2A
40	AKR1B1	112	Bassoon	184	CD155	256	CPN1	328	EEF2	400	FUCA2	472	Histone H2A.Z
41	AKR1C3	113	BAZ2B	185	CD157	257	CPNE3	329	EFEMP2	401	FAH	473	Histone H2B K
42	AKR7A2	114	BCHE	186	CD16	258	CPS1	330	EFTUD2	402	G0/G1switch 2	474	Histone H3.3
43	ALAD	115	Bcl-w	187	CD21	259	CKMM	331	EHD1	403	G3BP	475	Histone H4
44	ALT	116	BCOR	188	CD32	260	CRF21	332	EHD3	404	GALNT2	476	HLA-C
45	ADH	117	beta 1 Spectrin	189	CD35	261	CRHBP	333	EIF3S2	405	gamma Catenin	477	HMGB1
46	AOX1	118	CRYBB1	190	CD39L4	262	CrkL	334	eIF4A1	406	GAPDH	478	HMGB2
47	ALDH16A1	119	beta 1 Tubulin	191	CD41	263	CRMP2	335	eIF5A	407	GARNL1	479	HMGB3
48	ALDH1A1	120	CUBB3	192	CD42b	264	CRTAC1	336	ELAVL1	408	GART	480	HMGN2
49	ALDH9A1	121	BID	193	CD48	265	CS	337	EMILIN1	409	Gastrokine 1	481	HN1
50	ALKP	122	BIN2	194	CD5L	266	Ctip2	338	EMSY	410	GATM	482	FoxA1
51	ALP	123	BIRC6	195	CD9	267	Cux2	339	EN2	411	GBE1	483	hnRNP A1
52	MAN1A1	124	BLMH	196	CD98	268	Cyclophilin A	340	Endorepellin	412	GCDFP 15	484	hnRNP A2B1
53	alpha Actinin 4	125	BLVRB	197	CDA	269	Cyclophilin B	341	ENO1	413	GCLC	485	hnRNP C1+C2
54	Alpha Fodrin	126	BMP-1	198	CDC5L	270	Cystatin D	342	ENO1+ENO2+ENO3	414	GCSH	486	hnRNP G
55	alpha Glucosidase II	127	BPGM	199	CDK2	271	Cystatin E	343	ENSA	415	GDA	487	hnRNP L
56	alpha-Synuclein	128	BPIFB1	200	CEACAM-8	272	Cystatin S	344	Envoplakin	416	GDF7	488	hnRNP M1-M4
57	alpha Tubulin	129	BPIL1	201	CECR1	273	Cystatin SN	345	EDN	417	GDI1	489	hnRNP U
58	CRYAA	130	BRCA 2	202	CENPF	274	CSRP1	346	EPB41	418	GDI2	490	Hornerin
59	ALS	131	BRD2	203	CEP57	275	CYTL1	347	EPCR	419	Gephyrin	491	Hoxb3
60	Als2	132	Brevican	204	CES1	276	Cytochrome b5	348	Ephrin B1	420	GFAP	492	HOXD11
61	ALS2CR1	133	Brg1	205	CETP	277	Cytochrome c (n)	349	Ephrin B2	421	GHRF	493	HP1BP3
62	Aminoacylase	134	BRSK1	206	Cezanne	278	Cytokeratin 1	350	EPHX2	422	GIP	494	HPD
63	Androgen Receptor	135	BTD	207	CFHR1	279	Cytokeratin 10	351	EPPK1	423	GLIPR2	495	HPR
64	ANGPTL6	136	BTF3	208	CFHR4	280	Cytokeratin 13	352	Eps15	424	GLRX1	496	HPRT
65	ANGPTL8	137	C1q	209	CFHR5	281	Cytokeratin 14	353	ERAB	425	G6PD	497	HRG
66	ANK	138	C1qA	210	CFI	282	Cytokeratin 15	354	ERAP2	426	PRKCSH	498	HRSP12
67	Ankrd26	139	C1qB	211	CFL1	283	Cytokeratin 16	355	ERp29	427	GLUD1	499	HSC70
68	Annexin A1	140	C1qR1	212	CFVII	284	Cytokeratin 17	356	ERp57	428	CGH	500	HSP47
69	Annexin A2	141	C1RL	213	CHC17	285	Cytokeratin 20	357	ERp72	429	GSTO1		
70	Annexin A6	142	C1s	214	Chitobiase	286	Cytokeratin 3	358	ESD	430	GSS		
71	Annexin V	143	ELP6	215	Chitotriosidase	287	Cytokeratin 4	359	ESR1	431	GPD1		
72	ANP	144	C4.4A	216	CHORDC1	288	Cytokeratin 5	360	ETL	432	Glycoprotein V		

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.