

RayBio® Human Protein S-Acylation Antibody Array 2

For Simultaneously Detecting the Relative Levels of
S-Acylation (or S-Palmitoylation) of 493 Human Proteins

User Manual
Revised Aug. 19th, 2024

Cat#: AAH-ACYL-G2-4 (4 Sample Kit)

Cat#: AAH-ACYL-G2-8 (8 Sample Kit)

Please read manual carefully
before starting experiment





RayBio® Human Protein S-Acylation Antibody Array 2 Protocol

TABLE OF CONTENTS

I.	Introduction.....	2
II.	Materials Provided.....	3
III.	Additional Materials Required.....	4
IV.	Reagent Preparation.....	5
V.	Overview and General Considerations.....	5
	A. Preparation of Samples.....	5
	B. Handling Glass Slides.....	6
	C. Incubation.....	6
VI.	Protocol.....	7
	A. Dry the Array Slides.....	7
	B. Blocking and Incubation.....	7
	C. Fluorescence Detection.....	9
VII.	Interpretation of Results.....	10
VIII.	Troubleshooting Guide.....	13
IX.	Reference List.....	14

I. Introduction

Protein S-acylation (or protein S-palmitoylation) plays an unusually prominent role in cell signaling, development and growth. RayBio® Human Protein S-Acylation Antibody Array 2 is specifically designed for simultaneous identification of the relative levels of S-Acylation of cysteine of 493 different human proteins in cell lysate, culture supernatant, serum, plasma and other biological samples. By monitoring the changes in protein S-Acylation 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.

The array kit uses a modified 'biotin-switch' (acyl-biotinyl exchange (ABE)) method (Jaffrey *et al.*) to allow for the direct visualization of S-acylated proteins on the antibody array. Prior to running the array, the samples are labeled. Unmodified free cysteines on proteins in the sample are first blocked. S-acylated cysteines are then selectively reduced for specific labeling with biotin-maleimide reagents, which irreversibly bind to the cysteine thiol that was S-acylated. Biotinylation of the newly formed thiol groups can then be detected on the antibody array. If desired, avidin resin can be used to selectively enrich S-acylated proteins/peptides labeled with biotin. The biotin labeled sample is added into antibody array glass slide wells. The antibody array slide wells are washed. 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. Materials Provided

Store kit at $\leq -20^{\circ}\text{C}$ immediately upon arrival. Kit must be used within the 6 months expiration date.

ITEM	COMPONENT	AAH-ACYL-G2-4	AAH-ACYL-G2-8	STORAGE TEMPERATURE AFTER THAWING**
1	RayBio Glass Slide*	1	2	≤ -20 °C
2	Blocking Buffer	1 bottle (8 ml)	2 bottles (8 ml)	
4	Fluorescent Dye-Conjugated Streptavidin (Cy3 equivalent)	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°C
9	Protease Inhibitor Cocktail	1 vial		≤ -20 °C
10	S-Acylation Buffer A	12 ml		RT
11	S-Acylation Buffer B	3 ml		RT
12	S-Acylation Buffer C	25 ml		RT
13	S-Acylation Buffer D	12 ml		RT
14	S-Acylation Buffer E	12 ml		RT
15	S-Acylation Blocking Reagent	2 vials, enough for 2 separate experiments. Crystalline solid.		Prepare immediately prior to use. Do not store.
16	S-Acylation Reduction Reagent	2 bottles, enough for 2 separate experiments. Crystalline solid.		Prepare immediately prior to use. Do not store.
17	S-Acylation Labeling Reagent	2 vials, enough for 2 separate experiments. Crystalline solid.		Prepare immediately prior to use. Do not store.
Other Kit Components: Adhesive film				

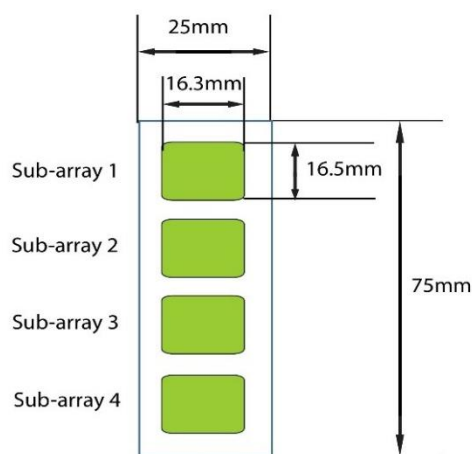
*Each slide contains 4 identical subarrays

**For up to 3 months (unless stated otherwise) or until expiration date

III. Additional Materials Required

- Acetone, $\geq 98\%$ (hazardous)
- 1.5 mL microcentrifuge tubes
- 15 mL tubes (polypropylene)
- 10 mL graduated cylinders (X2)
- Benchtop centrifuge and microcentrifuge (4°C)
- Precision pipettes to deliver $2\ \mu\text{L}$ to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- Water bath or heat block
- 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. **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 into 2 ml of the 1X Cell Lysis Buffer to prepare a 1X Cell Lysis Buffer with Protease Inhibitor Cocktail solution. Mix well before use.
3. **S-Acylation Blocking Reagent:** Make fresh. Spin briefly, add 50 µl Acylation Buffer B, vortex until all crystals are dissolved completely, then transfer everything into 5 ml S-Acylation Buffer A, mix well.
4. **S-Acylation Reduction Reagent:** Make fresh. Spin briefly, add 10mL S-Acylation Buffer C, vortex until all crystals are dissolved completely, mix well.
5. **S-Acylation Labeling Reagent:** Make fresh. Spin briefly, add 100 µL dH₂O, vortex until all crystals are dissolved completely.
6. **Acetone (not included):** pre-chilled (-20°C).
7. **4:1 acetone/water mixture:** 4 parts acetone mixed with 1 part dH₂O, pre-chilled (-20°C).
8. **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.
9. **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

1. Cell lysate preparation: 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 2×10^7 cells/ml in the 1X Cell Lysis Buffer with Protease 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. Lysates should be used immediately or aliquoted and stored at –80 °C. Thawed lysates should be kept on ice prior to use.

2. Biotinylation of S-acylated cysteines.

This kit contains enough reagent to label 40 samples containing 100-200 µg of total protein each.

1. Prepare 100 µl sample with total protein concentration at 1-2 mg/mL. It is recommended to label samples with equivalent protein concentrations.
2. Add 200 µl prepared S-Acylation Blocking Buffer (use fresh reagent, prepared immediately prior to use) into each sample. Incubate the samples in dark at 50 °C on a shaker with gentle rocking for 30 minutes.
3. Precipitate protein by adding 1200 µl (4 volume) pre-chilled (-20°C) acetone for each sample. Mix thoroughly by inversion followed by incubation at -20°C for 1 hour.
4. Centrifuge at 14,000 × g for 10 minutes at 4°C.
5. Carefully dispose of the supernatant, without dislodging the protein pellet.
6. Add 500 µl pre-chilled 4:1 acetone/water mixture to wash the pellet. Repeat steps 4 and 5.
7. Repeat step 6 to wash the pellet one more time.
8. Allow the acetone to evaporate from the uncapped tube at room temperature for 30 minutes. Do not over-dry pellet, or it may not be dissolved properly.
9. Reconstitute the pellet in 40 µl S-Acylation Buffer D.
10. Add 160 µl S-Acylation Reducing Buffer (use fresh reagent, prepared immediately prior to use), Incubate for 1 hour at 37 °C. (Option: At this step samples can incubate with reducing buffer at room temperature overnight for optimal result)
11. Add 4 µl S-Acylation Labeling Buffer (use fresh reagent, prepared immediately prior to use) to the reconstituted sample and incubate for 2 hours at room temperature with gentle rotation.
12. Repeat steps 3-5. Allow the acetone to evaporate from the uncapped tube at room temperature for 30 minutes. Do not over-dry pellet, or it may not be dissolved properly.
13. Reconstitute each protein pellet in 40 µl S-Acylation Buffer E. Vortex the tube several times and then quickly spin down (it is normal to have undissolved protein pellet). Transfer supernatant to new tubes. The sample is now ready for analysis by antibody array. The labeled sample can be stored at -20°C for future analysis.
14. Dilute each sample 10 folds with the antibody array blocking buffer (Item 2).

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.

- Incubation steps such as step 2 (sample incubation, page 10), or step 6 (Fluorescent dye-Conjugated Streptavidin incubation, page 11) 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 6 in page 11 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 Dilute each sample 10 folds with the antibody array blocking buffer (Item 2). **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.

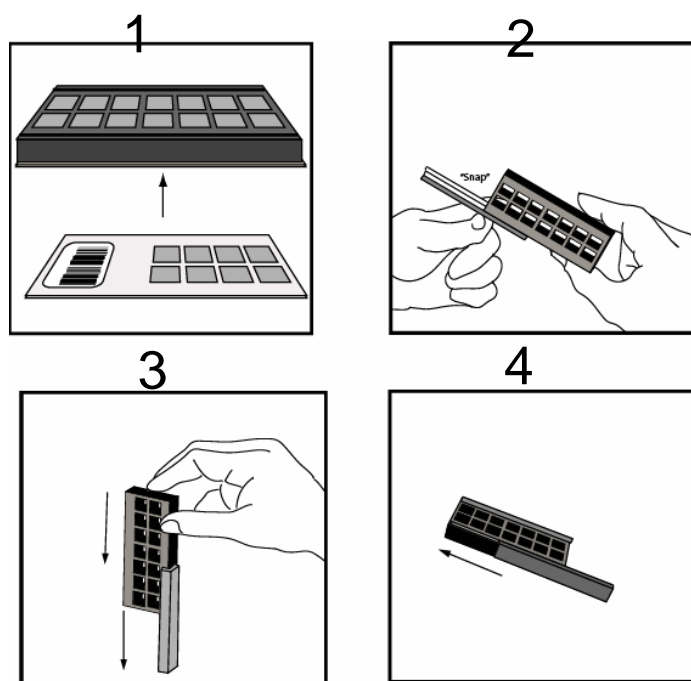
5. Decant the Wash Buffer I from each well. Put the Glass Slide with Frame into a box with Wash Buffer II (cover the whole glass slide and frame with Wash Buffer II), and wash 2 times, 5 min per wash, at room temperature with gentle shaking.
6. Remove all of Wash Buffer II from each well. Add 400 μ l 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.

7. Incubate at room temperature with gentle shaking for 2 hours in the dark.

Note: Incubation may be done at 4 °C overnight.

8. 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).
9. 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.



10. Wash the glass slide with 40 ml of Wash Buffer II for 5 min. Repeat one more time for a total of 2 washes.
11. 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 N₂ 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

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.

RayBio Human Protein S-Acylation 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
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	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	POS3	POS3	POS2	POS2	POS1	POS1

RayBio Human Protein S-Acylation Antibody Array 2 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	11b-HSD1	73	BLAME	145	C-peptide	217	FoxO1	289	KIF3B	361	PTH	433	Serpin G1
2	2B4	74	BMP-9	146	Creatinine	218	FoxP3	290	KLF4	362	Troponin C	434	SERTAD2
3	4-1BB	75	BMX	147	CRP	219	FRK	291	LAG-3	363	PDX-1	435	SHBG
4	ABL1	76	BNIP2	148	CRTAM	220	ARB1	292	pro-Glucagon	364	PDF	436	SMAC
5	ACE	77	Btk	149	CSH1	221	Furin	293	Layilin	365	PEPSINOGEN I	437	SNCG
6	ACE-2	78	ApoC1	150	gamma-Thrombin	222	Fyn	294	LDL R	366	PEPSINOGEN II	438	SSTR5
7	ACK1	79	CA9	151	CutA	223	GADD45A	295	Legumain	367	Vasopressin	439	SCGF
8	ACPP	80	CA15-3	152	Troponin T	224	Galectin-1	296	LH	368	PGPR-5	440	SOST
9	ACTH	81	CA19-9	153	Cyclin D1	225	Galectin-3BP	297	LIMPII	369	PI 16	441	SOX17
10	ADAM-9	82	CA125	154	Cystatin A	226	Galectin-7	298	LIN41	370	PIK3R1	442	SOX2
11	Neurokinin A	83	Cadherin-13	155	Cystatin B	227	Gas1	299	Livin	371	PIM2	443	SPARCL1
12	ADAMTS1	84	CLEC14A	156	Cystatin C	228	Gastrin	300	LOX-1	372	PKM2	444	SPINK1
13	ADAMTSL2	85	Calbindin D	157	Cytochrome C (d)	229	GATA-3	301	LPS	373	Plasminogen	445	SRMS
14	ADAMTS4	86	Calcitonin	158	Cytokeratin 8	230	GATA-4	302	LRG1	374	Podocalyxin	446	SSEA-1
15	ADAMTS5	87	Calreticulin	159	Cytokeratin 18	231	Gelsolin	303	LTf	375	POMC	447	SSEA-4
16	ADAMTS10	88	Calsyntenin-1	160	Cytokeratin 19	232	Ghrelin	304	LTK	376	PON1	448	SSTR2
17	ADAMTS13	89	CPN2	161	DBI	233	GLP-1	305	Lumican	377	PON2	449	Survivin
18	ADAMTS15	90	CART	162	DCBLD2	234	GPI	306	Lyn	378	PPARG2	450	SYK
19	ADAMTS17	91	Caspase-3	163	D-Dimer	235	GPBB	307	LYRIC	379	PP2R5C	451	Syndecan-1
20	ADAMTS18	92	Caspase-8	164	DEFA1/3	236	GMNN	308	LYVE-1	380	NR3C3	452	Syndecan-3
21	ADAMTS19	93	Cathepsin B	165	CPA1	237	GPR-39	309	LZTS1	381	INSL3	453	TACE
22	Adipsin	94	Cathepsin D	166	Desmin	238	GPX1	310	Mammaglobin A	382	Pro-BDNF	454	TAF4
23	Afamin	95	Cathepsin L	167	DLL1	239	GPX3	311	Marapsin	383	Procalcitonin	455	Tyk2
24	AFP	96	Cathepsin S	168	DLL4	240	Pancreastatin	312	MATK	384	Pro-Cathepsin B	456	Tec
25	ALBUMIN	97	CBP	169	DMP-1	241	GRP	313	MBL	385	Thrombin	457	TFF3
26	IL-28B	98	CCK	170	DPPIV	242	GRP75	314	C1qTNF1	386	Prohibitin	458	Thrombomodulin
27	Aldolase A	99	CD23	171	BNP	243	GRP78	315	Mer	387	ProSAA5	459	TK1
28	Aldolase B	100	CD24	172	E-Cadherin	244	GSR	316	Mesothelin	388	Prostasin	460	Thyroglobulin
29	Aldolase C	101	CD36	173	Endorphin Beta	245	GST	317	MICB	389	PSP	461	TIM-1
30	ALK	102	CD38	174	EDNRA	246	HADHA	318	Midkine	390	Pro-MMP-7	462	TNK1
31	Alpha Lactalbumin	103	CD44	175	Enolase 2	247	HAI-1	319	MINA	391	Pro-MMP-9	463	TOPORS
32	Alpha 1 AG	104	CD45	176	ENPP2	248	HAI-2	320	FABP3	392	Protein p65	464	TPA
33	A1BG	105	CD46	177	EpCAM	249	hCG alpha	321	MSHa	393	PSA-Free	465	TRA-1-60
34	A1M	106	CD47	178	EphA1	250	hCgb	322	MTUS1	394	PSA-total	466	TRA-1-81
35	A2M	107	CD55	179	EphA2	251	Hck	323	Myoglobin	395	PTHLP	467	Transferrin
36	TPM1	108	CD59	180	EphA3	252	He4	324	NAIP	396	PTN	468	Trappin-2
37	ALPP	109	CD71	181	EphA4	253	Hemopexin	325	Nanog	397	PTPRD	469	TRKB
38	Pro-MMP-13	110	CD74	182	EphA5	254	Hepcidin	326	NELL2	398	PYK2	470	Troponin I
39	AMICA	111	CD90	183	EphA6	255	HSP32	327	Neprilysin	399	PYY	471	TYRO10
40	AMPKa1	112	CD97	184	EphA7	256	HoxA10	328	Galanin	400	Ras	472	TRPC1
41	Amylin	113	CD79 alpha	185	EphA8	257	Haptoglobin	329	Nesfatin	401	RBP4	473	TRPC6
42	ANGPTL3	114	CD200	186	EphB1	258	HSP10	330	Nestin	402	RECK	474	TRPM7
43	ANGPTL4	115	CEA	187	EphB2	259	HSP20	331	NET1	403	RELM alpha	475	Trypsin 1
44	Annexin A7	116	CEACAM-1	188	EphB3	260	HSP27	332	Netrin G2	404	Resistin	476	TSH
45	APC	117	Ceruloplasmin	189	EphB4	261	HSP40	333	Netrin-4	405	RET	477	TSLP
46	APCS	118	CFHR2	190	EphB6	262	HSP60	334	Neuropeptide Y	406	RIP1	478	TXX
47	Apelin	119	Chemerin	191	ERRa	263	HSP70	335	NF1	407	ROCK1	479	Uromodulin
48	Apex1	120	CHI3L1	192	Erythropoietin R	264	HSP90	336	NM23-H1/H2	408	ROCK2	480	TFF1
49	APN	121	Chromogranin A	193	ESAM	265	HSPA8	337	Presenilin 2	409	ROR1	481	VDUP-1
50	ApoA1	122	Chymase	194	EV15L	266	HTRA2	338	Notch-1	410	ROR2	482	VEGF R1
51	ApoA2	123	cIAP-2	195	EXTL2	267	IBSP	339	NPTX1	411	ROS	483	VGf
52	ApoA4	124	Ck beta 8-1	196	FABP1	268	IGF2BP1	340	NPTXR	412	RYK	484	VIPR2
53	ApoB	125	CKMB	197	FABP2	269	IGFBP-5	341	Progesterone	413	S100A4	485	VDR
54	ApoC2	126	Claudin-3	198	FABP4	270	IDUA	342	Ntn1	414	S100A6	486	VDB
55	ApoB100	127	Claudin-4	199	FAK	271	IL-33	343	OCT3/4	415	S100A8	487	PROS1
56	ApoE	128	CLEC3B	200	FAP	272	IL-34	344	Omentin	416	S-100b	488	Vitronectin
57	ApoE3	129	Clusterin	201	Fcg RIIB/C	273	INSRR	345	Osteocalcin	417	SART1	489	VWF
58	ApoD	130	CNDP1	202	Fen-1	274	ITGAV	346	Osteopontin	418	SART3	490	WT1
59	ApoM	131	Fc gamma RIIB	203	FER	275	CD61	347	OX40	419	SCG3	491	XIAP
60	ApoH	132	Factor XIII B	204	Ferritin	276	Itk	348	p21	420	Selenoprotein P	492	ZAG
61	APP	133	COCO	205	Fetuin A	277	ITM2B	349	p27	421	SEMA3A	493	ZAP70
62	ASPH	134	C2	206	Fetuin B	278	Kallikrein 2	350	p53	422	Serotonin		
63	Attractin	135	C3a	207	FGFR1	279	ApoC3	351	PAI-1	423	Serpin A1		
64	B3GNT1	136	C5a	208	FGFR1 alpha	280	Kallikrein 5	352	PAK7	424	Serpin A12		
65	BAF57	137	C7	209	FGFR2	281	Kallikrein 6	353	Pappalysin-1	425	Serpin A3		
66	BAFF	138	C8b	210	Fibrinogen	282	Kallikrein 7	354	PP	426	Serpin A4		
67	BAI-1	139	C9	211	Fibrinopeptide A	283	Kallikrein 8	355	Presenilin 1	427	Serpin A5		
68	BCAM	140	CFH	212	Fibronectin	284	Kallikrein 10	356	PARK7	428	Serpin A8		
69	B2M	141	Contactin-1	213	Ficolin-3	285	Kallikrein 11	357	Visfatin	429	Serpin A9		
70	Beta Defensin 4	142	Contactin-2	214	FIH	286	Kallikrein 14	358	P-Cadherin	430	Serpin B5		
71	Beta IG-H3	143	CBG	215	FOLR1	287	KCC3	359	PCAF	431	Serpin D1		
72	Biglycan	144	COX-2	216	FOXN3	288	KCTD10	360	PD-1	432	Serpin I1		

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 protein	Make sure to use the correct amount of protein
	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 prostaglandin 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 products 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.