RayBio® G-Series Human Protein S-Nitrosylation Antibody Array 2

For Simultaneously Detecting the Relative Level of S-Nitrosylation of Human Protein

User Manual

(Revised Mar. 22nd, 2024)

Cat#: AAH-SNO-G2-4 (4 Sample Kit)
Cat#: AAH-SNO-G2-8 (8 Sample Kit)

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RayBiotech Life, Inc.

RayBio® G-Series Human Protein S-Nitrosylation Antibody Array 2 Protocol

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I. Introduction

Protein S-Nitrosylation plays an unusually prominent role in cell signaling, development and growth. RayBio® Human Protein S-Nitrosylation Antibody Array 2 is specifically designed for simultaneous identification of the relative levels of S-Nitrosylation 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-Nitrosylation 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.

RayBio® S-Nitrosylation Detection Kit uses a modified 'biotin-switch' method (Jaffrey et al.) to allow for the direct visualization of S-nitrosylated proteins by western blot analysis as well as by antibody array. In this S-nitrosylation biotin switch assay, unmodified free cysteines are first blocked. S-nitrosylated cysteines are then selectively reduced for specific labeling with biotin-maleimide reagents, which irreversibly bind to the cysteine thiol that was S-nitrosylated. Biotinylation of the newly formed thiol groups can then be detected by western blot or antibody array. In addition, avidin resin can be used to selectively enrich S-nitrosylated 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 ≤ -20 °C immediately upon arrival. Kit must be used within the 6 months expiration date.

ITEM	COMPONENT	AAH-SNO-G2-4	AAH-SNO-G2-8	STORAGE TEMPERATURE AFTER THAWING**
1	RayBio® Glass Slide*	1	2	≤-20°C
2	Blocking Buffer	1 bottle (8ml/ea)	2 bottles (8ml/ea)	
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 ℃
9	Protease Inhibitor Cocktail	1 vial		≤-20°C
10	S-Nitrosylation Buffer A	12 ml		RT
11	S-Nitrosylation Buffer B	3 ml		RT
12	S-Nitrosylation Buffer C	5 ml		RT
13	S-Nitrosylation Buffer D	3 ml		RT
14	S-Nitrosylation Buffer E	12 ml		RT
15	S-Nitrosylation Buffer F	12 ml		RT
16	S-Nitrosylation Blocking Reagent	2 vials, enough for 2 separate experiments. Crystalline solid.		Prepare immediately prior to use. Do not store.
17	S-Nitrosylation Reduction Reagent I	2 vials, enough for 2 separate experiments. Crystalline solid.		Prepare immediately prior to use. Do not store.
18	S-Nitrosylation Reduction Reagent II	2 vials, enough for 2 separate experiments. Crystalline solid.		Prepare immediately prior to use. Do not store.
19	S-Nitrosylation Labeling Reagent	2 vials, enough for 2 separate experiments. Crystalline solid.		Prepare immediately prior to use. Do not store.
Other Kit Components: Adhesive film				

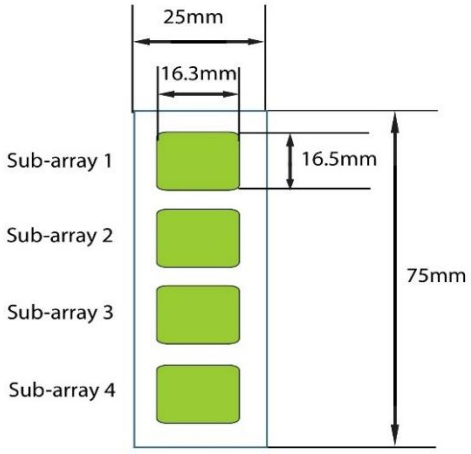
*Each slide contains 4 identical subarrays

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

III. Additional Materials Required

- Acetone, ≥ 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 µl 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-Nitrosylation Blocking Reagent:** Make fresh. Spin briefly, add 50 µl S-Nitrosylation Buffer B, vortex until all crystals are dissolved completely, then transfer everything into 5 ml S-Nitrosylation Buffer A, mix well.
4. **S-Nitrosylation Reduction Reagent I:** Make fresh. Spin briefly, add 800 µl S-Nitrosylation Buffer C, vortex until all crystals are dissolved completely.
5. **S-Nitrosylation Reduction Reagent II:** Make fresh. Spin briefly, add 500 µl S-Nitrosylation Buffer D, vortex until all crystals are dissolved completely. Take add 4 µl dissolved S-Nitrosylation Reduction Reagent II into 800 µl dissolved S-Nitrosylation Reduction Reagent I, mix well. This is S-Nitrosylation Reducing Buffer.
6. **S-Nitrosylation Labeling Reagent:** Make fresh. Spin briefly, add 100 µL dH2O, vortex until all crystals are dissolved completely.
7. **Acetone (not included):** pre-chilled (-20°C).
8. **4:1 acetone/water mixture:** 4 parts acetone mixed with 1 part dH2O, pre-chilled (-20°C).
9. **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.
10. **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 $2x10^7$ 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-nitrosylated 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-Nitrosylation 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 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 100 µl S-Nitrosylation Buffer E.
10) Add 22 µl S-Nitrosylation Reducing Buffer (use fresh reagent, prepared immediately prior to use), along with 3 µl S-Nitrosylation 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.
11) 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.
12) Reconstitute each protein pellet in 40 µl S-Nitrosylation Buffer F. 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.
13) 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.

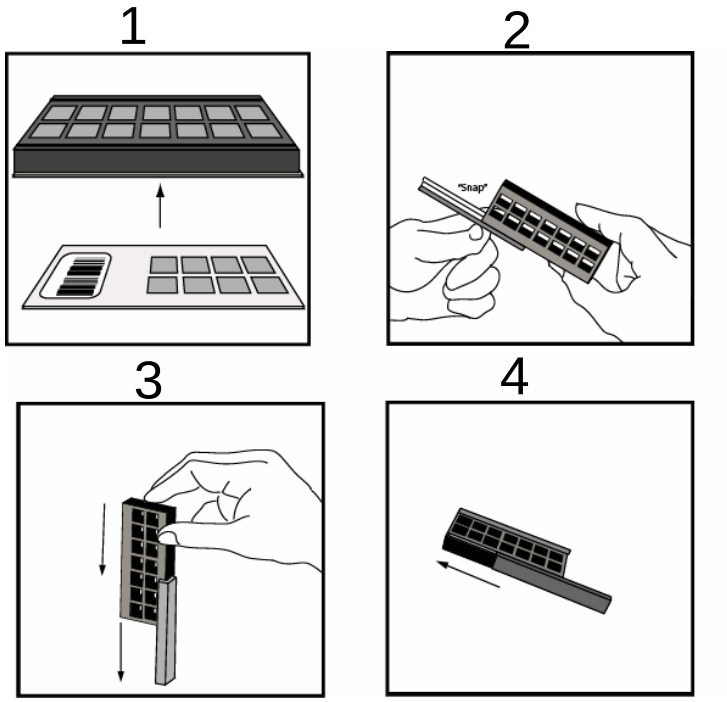
6. Remove all of Wash Buffer II from each well. Add 400 µl of the 1X Fluorescent dye-Conjugated Streptavidin solution to each subarray. Cover the incubation chamber with the Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in a dark room.

   Note: Avoid exposing the array slide to light from this step forward.

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 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

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® G-Series Human Protein S-Nitrosylation 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
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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® G-Series Human Protein S-Nitrosylation 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	PEDF	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	PGRP-S	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	PPP2R5C	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	ProSAAS	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	TXK
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 RIIIB	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

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