RayBio® Biotin Label-based Human Antibody Array 1

For the Simultaneous Detection of the Expression Levels of 507 Human Proteins in Cell Culture Supernates, Serum and Plasma.

User Manual

(Revised Apr 1, 2009)

(Cat#: AAH-BLG-1-2; AAH-BLG-1-4)

RayBiotech, Inc.

As the Protein Array Pioneer Company, Excellence and Innovation Is Our Goal

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RayBiotech, Inc

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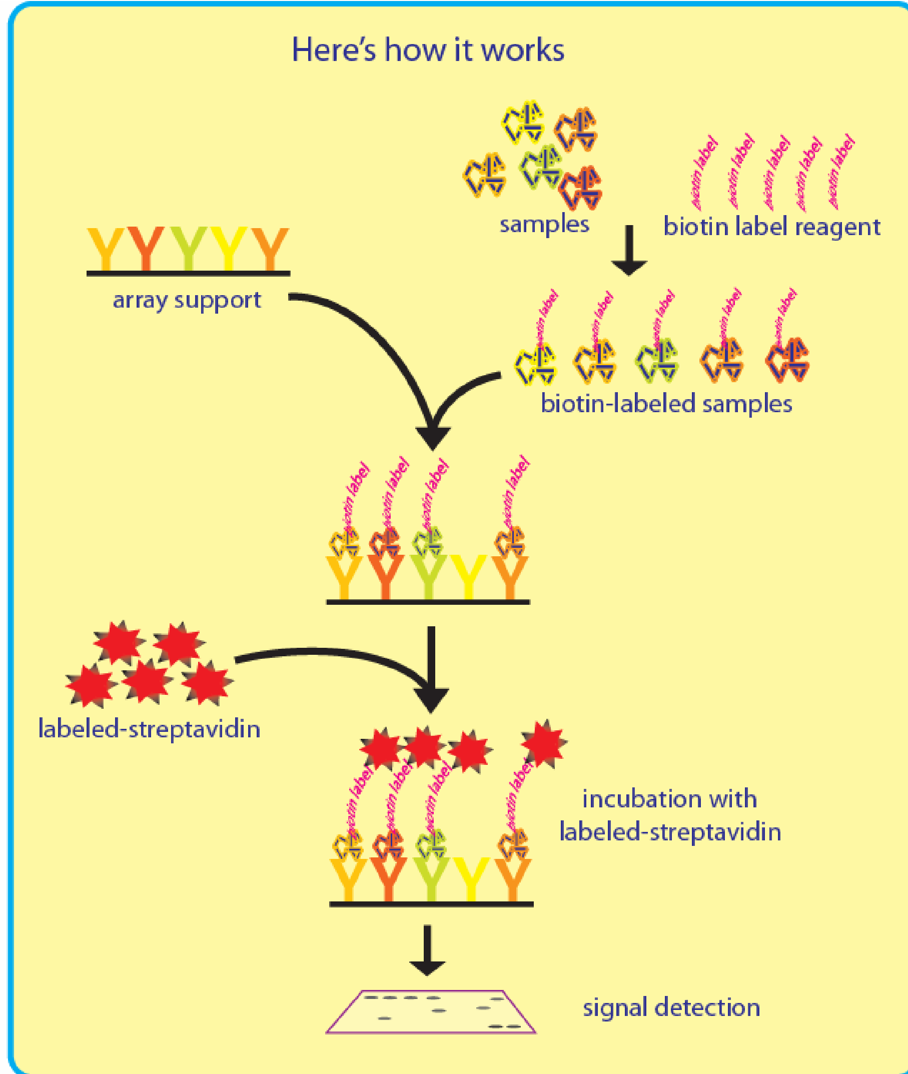
I. Introduction

Recent technological advances by Raybiotech have enabled the largest commercially available antibody array to date. With the L Series 507, researchers can now obtain a broad, panoramic view of cytokine expression. The expression levels of 507 human target proteins can be simultaneously detected, including cytokines, chemokines, adipokine, growth factors, angiogenic factors, proteases, soluble receptors, sobuble adhesion molecules and other proteins in cell culture supernate, serum and plasma. Furthermore, an internal control is used to monitor the whole process including biotin-labeling, so this massive array will accurately reflect the available cytokines in your sample.

The first step in using the RayBio® Biotin label-based human antibody array 1 is to biotinylate the primary amine of the proteins in cell culture supernates, serum and plasma. The biotin-labeled sample is then added onto glass chip and incubated at room temperature. Fluorescent dye-Conjugated Streptavidin (cy3 equivalent) is used to visualize the signals.



II. Materials Provided

Upon receipt, the kit should be stored at -20 °C. Please use within 6 months from the date of shipment. After initial use, the Blocking Buffer, Stop Solution, 20X Wash Buffer I and II, Serum Buffer and Fluorescent dye-Conjugated Streptavidin should be

stored at 4 °C to avoid repeated freeze-thaw cycles. The Array I Glass Chip and internal control should be kept at -20 °C.

- Dialysis tube (Item A, 4 tubes for 2-subarrary chips, and 8 for 4-subarray chips)
- Labeling Reagent (Item B, 1 tube for 2-subarrary chips, and 2 for 4-subarray chips)
- Internal control (Item C, 1 tube for 2-subarrary chips, and 2 for 4-subarray chips)
- Stop Solution (Item D, 50 µl)
- RayBio® Biotin label-based human antibody array 1 Glass Chip with Frame (Item E, each slide with 2 Subarrays, 1 slide for 2 subarray chips, and 2 for 4 subarray chips)
- Blocking Buffer (Item F, 8 ml)
- 20X Wash Buffer I (Item G, 30ml)
- 20X Wash Buffer II (Item H, 30ml)
- Fluorescent dye-Conjugated Streptavidin (Item I, cy3 equivalent, 1 tube for 2-subarrary chips, and 2 for 4-subarray chips)
- Adhesive film (Item J)
- Serum Buffer (Item K, 8 ml)
- D-Tube Floating Rack
- 30 ml centrifuge tube

III. Additional Materials Required

- 1X PBS, pH=8.0
- Shaker and plastic or glass box
- 1 ml tube
- Laser scanner for fluorescence detection

- Aluminum foil
- Distilled water

IV. Overview and General Considerations

A. Handling glass chips

- The microarray slides are sensitive, so do not touch the array surface by tips, forceps or hand. Hold the slides by the edges only.
- Handle the slides with latex free gloves.
- Avoid breaking the glass slide.
- A clean environment is essential
- Remove the final buffer by gently applying suction with a pipette to corners of each chamber. Do not touch the array, only the sides.

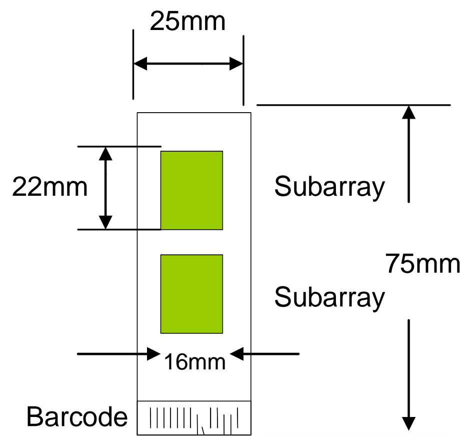
B. Incubation of Antibody Array

- Completely cover the array area with sample or buffer during incubation, and cover the incubation chamber with adhesive film or a plastic sheet protector to avoid drying, particularly when incubation is more than 2 hours.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.

- Avoid cross-contamination by preventing overflow to neighboring wells.
- Several incubation steps such as step 2 (sample incubation) in page 10, or step 7 (Fluorescent dye-Conjugated Streptavidin incubation) in page 11 may be done at 4 °C for overnight. Please make sure to cover the incubation chamber tightly to prevent evaporation.
- Avoid array slide exposure to light since step 6 in page 11.

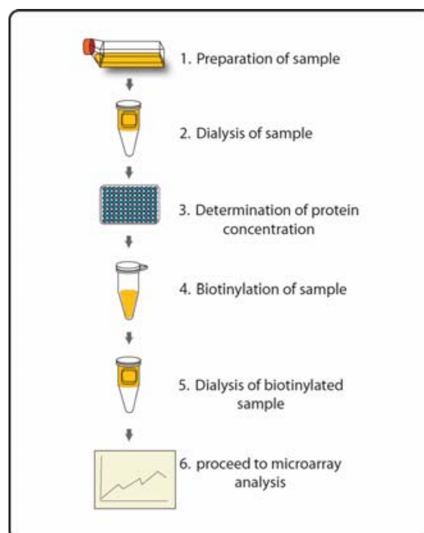
V. Protocol

Layout of Array Glass Chip

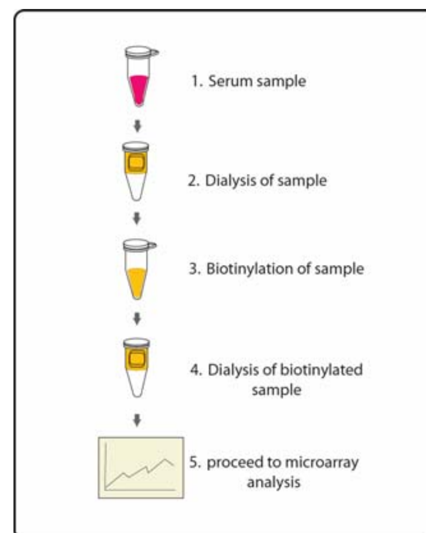


Assay Diagram

1. Cell Culture Supernates



2. Serum or plasma



A. Preparation of Cell Culture Supernates

The cell culture supernates can be prepared in the following conventional manner:

To prepare cell culture supernates (cell conditioned media), cells are plated in 100 mm tissue culture dishes at a density of $1x10^6$ cells (*) per dish. The cells are then cultured with complete culture medium for 24~48 hours (**). The complete culture medium is replaced with lower serum medium such as 0.2% FCS serum, and then the cells are cultured for 48 hour (**) again once more. The supernates are collected, centrifuged at 1,000 g for 10 min, aliquoted and stored at –80 ^{0}C until use. Meanwhile, the cells are also collected and the total protein concentration is determined. For each sample it is recommended that the concentrations of the supernates and cell lysates (help nomalize different cell culture supernates) are determined using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227).

*Note: * The density of cells per dish used is dependent on the cell type More or less cells may be used.*

*** The culture time may be different and depends on your cell lines and research.*

B. Dialysis of Sample

The cell culture supernates, serum or plasma should be dialyzed with a Dialysis tube (Item A) before the biotin-labeling procedure. We recommend loading 200 µl cell culture supernates or 100 µl 5-fold diluted serum/plasma with 1X PBS (pH=8) (20 µl serum or plasma + 80 µl 1X PBS) into a dialyzer and dialyzing with

at least 500 ml 1X PBS buffer (pH= 8) at 4 ^{0}C. Change the 1X PBS buffer and dialyze again. Allow at least 3 h for each dialysis step, stir gently. The sample total volume may be changed after dialysis.

Note: Preparation of 1X PBS, pH=8.0, 0.6 g KCl, 24 g NaCl, 0.6 g KH_2PO_4, 3.45 g Na2HPO4 dissolve in 2500 ml deionized or distilled water. Adjust pH=8.0 with 1M NaOH and adjust final volume to 3000 ml with deionized or distilled water.

C. Biotin-labeling Sample

Avoid contamination with any solution containing amines (i.e., Tris, glycine) as well as Azide during the biotinylation process.

1. Briefly spin down Internal Control tube (Item C) before use. Add 100 µl 1X PBS, pH=8.0 into the Internal Control tube, pipette up and down to dissolve the powder.

   a) For labeling cell culture supernates: transfer 180 µl dialyzed sample into a new tube. Add 40 µl prepared Internal Control into the tube. Mix well.

   b) For labeling serum or plasma: add 40 µl prepared Internal Control into a new tube containing 35 µl dialyzed serum or plasma sample and 155 µl Serum Buffer (Item K).

   Note: Check serum total volume has been changed or not after dialysis step, and then adjust adding dialyzed serum and Serum buffer volume to keep same serum concentration

in Biotin-labeling step. For example, you need to use 70 µl dialyzed serum and 120 µl serum buffer if serum volume become 200 µl from 100 µl after dialysis.

2. Immediately before use, briefly spin down the Labeling Reagent tube (Item B). Add 100 µl 1X PBS into the tube, pipette up and down or vortex slightly to dissolve the powder to prepare 1X Labeling Reagent Solution.

3. Add an appropriate amount* of prepared Labeling Reagent into above tube with sample in step 2, mix well immediately. Incubate the reaction solution at room temperature with gentle shaking for 30 min. Gently tap the tube to mix the reaction solution every 5 min.

* *For labeling cell culture supernates: 36 µl of 1X Labeling Reagent Soluition for labeling 1 mg total protein in the cell culture supernates.*
Note: You need to re-calculate the total protein concentration if cell culture supernatet volume is changed after dialysis and you measure the total protein concentration before dialysis step.

For labeling serum or plasma: Add 22 µl of Labeling Reagent Solution into each prepared serum or plasma sample in page 8 step 1.

4. Add 3 µl Stop Solution (Item D) into above reaction solution and immediately dialyze as directed in Step B.

5. Samples should be centrifuged at 10,000 g for 5 min (4°C) after Dialysis.

Note: Samples (supernates) can be stored at -20 or -80 °C until microarray assay.

D. Dry the Glass Chip

Open the box containing glass chip, and take it out, and then let it air dry for 1 hour in a clean environment before use.

Note: Protect the chip from dust or others contaminants.

E. Blocking and Incubation of Antibody Array

1. Add 400 µl of Blocking Buffer (Item F) into each well (Glass Chip with Frame, Item E) and incubate at room temperature for 30 min to block slides. Make sure there are no bubbles are in the well.

2. Decant Blocking Buffer from each well (make sure to remove all of buffer). Add 400 µl of each sample into appropriate wells. Incubate arrays with sample at room temperature for 2 hours with gentle shaking or 4 °C for overnight.

Note: a. We recommended dilution of the biotin-labeled cell culture supernates 2-10 fold or the biotin-labeled serum/plasma 40-fold with blocking buffer. Make sure there are no bubbles in the wells.

b. The dilution used depends on the abundance of target proteins. The sample can be diluted further if the background or signal is too strong. If the signal is too weak, more sample can be used.

c. Incubation may be done at 4 °C overnight.

d. Avoid the flow of sample into neighboring wells.

3. Decant the samples from each well, and wash 3 times with 800 µl of 1X Wash Buffer I (Item G) at room temperature with gentle shaking for 5 min per wash.

4. Put the glass chip with frame into a box with 1X Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash 2 times at room temperature with gentle shaking for 10 min per wash.

5. Decant the Wash Buffer I from each well, Put the glass chip with frame into the box with Wash Buffer II (cover the whole glass slide and frame with Wash Buffer II), and wash 2 times at room temperature with gentle shaking for 5 min.

6. Remove all of Wash Buffer II in the well. Add 400 µl of 1X Fluorescent dye-Conjugated Streptavidin (cy3 equivalent) to each subarray. Cover the incubation chamber with Adhesive film, and then cover the plate with aluminum foil to avoid exposure to light or incubate in dark room (avoid array slide exposure to light in the following steps 7, 8, 9, 10 and 11).

   Note: briefly spin down the Fluorescent dye-Conjugated Streptavidin (cy3 equivalent) (Item I) before use. Add 1000 µl of Blocking Buffer into the tube to prepare a Streptavidin Concentrate. Pipette up and down to mix gently (don't store the Concentrate for next day use). Add 200 µl of Streptavidin

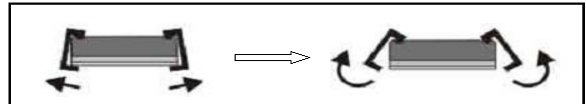
Concentrate into a tube with 800 µl of Blocking Buffer. Mix gently to prepare 1 X Streptavidin solution.

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

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

8. Decant the solution and disassemble the slide out of the incubation frame and chamber. Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket.

Note: Be careful not to touch the surface of the array side



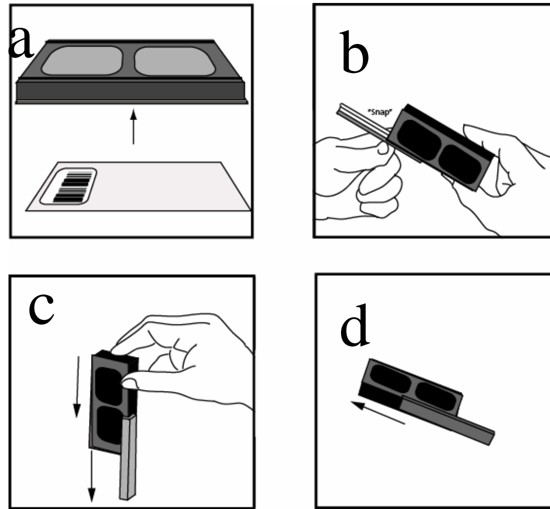
9. Gently put the glass chip into a 30 ml centrifuge tube prodided with 30 ml of 1X Wash Buffer I (add enough Wash Buffer I to cover the whole slide). Gently shake for 10 min. Remove the wash buffer. Repeat 2 times for a total of 3 washes.

10. Wash the glass chip with 30 ml of 1X Wash Buffer II. Repeat one time for a total of two washes for 5 min per wash.

11. Finally wash the glass chip with 30 ml of deionized or distilled water for 5 min.

Note: You may assemble the glass chip into an incubation chamber by following step if necessary as shown in the figure B. You may want to practice assembling the device with a blank glass slide:

a. Apply slide to incubation chamber barcode facing upward as in step (a).
b. Gently snap one edge of a snap-on side as shown in step (b).
c. Gently press other of side against lab bench and push in direction shown in step (c)
d. Repeat with the other side.



F. Fluorescence Detection

Put the glass chip into a 30 ml centrifuge tube provided, and dry the glass chip by centrifuge at 1,000 rpm for 3 minutes, or, dry the glass chip by a compressed N_2 stream. You can also let the glass chip dry completely in a clean environment (protect from light). Make sure the slides are absolutely dry before the scanning procedure. The signals can be visualized with a laser scanner, such as the Axon GenePix, using the cy3 channel.

Note: We recommend scanning the slides right after the experiment. You also can store the slides at –20 °C in the dark for several days. If you do not have a laser

scanner,we can provide this service for you. Just simply send your slide to us and we will take care of it.

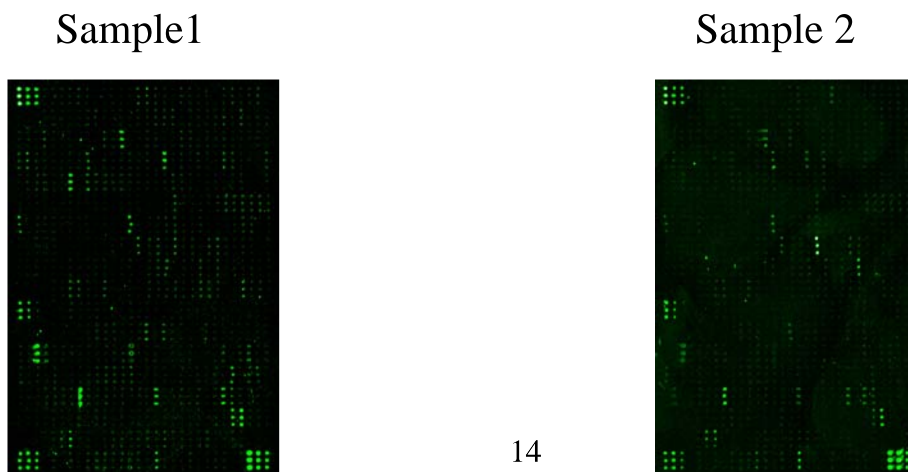
VI. Interpretation of Results:

The following figure shows the RayBio® **Biotin-label-based Human Antibody Array 1** probed with different cell culture supernates. The images were captured using a Axon GenePix laser scanner. A biotinylated protein and internal control will produce positive control signals, which can be used to identify the orientation and help normalize the results from different arrays being compared.

Antibody affinity varies significantly. The fluorescence intensity detected on the array with each antibody depends on its affinity; therefore, signal intensity comparison can be performed only within the same antibody/antigen system and not between different antibodies.

The **RayBio® Analysis Tool** is a program specifically designed for analysis of RayBio® Biotin Label-based Antibody Arrays. This tool will not only assist in compiling and organizing your data, but also reduces your calculations to a "copy and paste." Call RayBiotech, Inc. at 770-729-2992 for ordering information.

Sample1 Sample 2



RayBio® Biotin Label-based Human Antibody Array 1

P-1a	P-1b	P-1c	N	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
P-1a	P-1b	P-1c	N	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
P-1a	P-1b	P-1c	N	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
N	N	N	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
N	N	N	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
N	N	N	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
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91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
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IC-1a	IC-1b	IC-1c	B	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330
IC-1a	IC-1b	IC-1c	B	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330
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B	B	B	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	N	N	N
B	B	B	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	N	N	N
P-2a	P-2b	P-2c	B	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	N	IC-2a	IC-2b	IC-2c
P-2a	P-2b	P-2c	B	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	N	IC-2a	IC-2b	IC-2c
P-2a	P-2b	P-2c	B	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	N	IC-2a	IC-2b	IC-2c

RayBio® Biotin Label-based Human Antibody List

No.	Antibody
1	Positive 1a
2	Positive 1b
3	Positive 1c
4	neg
5	6Ckine
6	Activin A
7	Activin B
8	Activin C
9	Activin RIA / ALK-2
10	Activin RIB / ALK-4
11	Activin RII A/B
12	Activin RIIA
13	Adiponectin / Acrp30
14	AgRP
15	ALCAM
16	Angiogenin
17	Angiopoietin-1
18	Angiopoietin-2
19	Angiopoietin-4
20	Angiopoietin-like 1
21	Angiopoietin-like 2
22	Angiopoietin-like Factor
23	Angiostatin
24	APJ
25	AR (Amphiregulin)
26	APRIL
27	Artemin
28	Axl
29	B7-1 /CD80
30	BAFF R / TNFRSF13C
31	neg
32	neg
33	neg
34	BCMA / TNFRSF17
35	BD-1
36	BDNF
37	beta-Catenin
38	beta-Defensin 2
39	beta-NGF
40	BIK
41	BLC / BCA-1 / CXCL13
42	BMP-2
43	BMP-3
44	BMP-3b / GDF-10
45	BMP-4
46	BMP-5
47	BMP-6
48	BMP-7
49	BMP-8
50	BMP-15
51	BMPR-IA / ALK-3
52	BMPR-IB / ALK-6
53	BMPR-II
54	BTC
55	Cardiotrophin-1 / CT-1
56	CCL14 / HCC-1 / HCC-3
57	CCL28 / VIC
58	CCR1
59	CCR2
60	CCR3
61	CCR4
62	CCR5
63	CCR6
64	CCR7
65	CCR8
66	CCR9
67	CD14
68	CD27 / TNFRSF7
69	CD30 / TNFRSF8
70	CD30 Ligand / TNFSF8
71	CD40 / TNFRSF5
72	CD40 Ligand / TNFSF5 /CD154
73	CD 163
74	Cerberus 1
75	Chem R23
76	Chordin-Like 1
77	Chordin-Like 2
78	Csk
79	CLC
80	CNTF
81	CNTF R alpha
82	Coagulation Factor III / Tissue
83	CRIM 1
84	Cripto-1
85	CRTH-2
86	Cryptic
87	CTACK / CCL27
88	CTGF / CCN2
89	CTLA-4 /CD152
90	CV-2 / Crossveinless-2
91	CXCL14 / BRAK
92	CXCL16
93	CXCR1 / IL-8 RA
94	CXCR2 / IL-8 RB
95	CXCR3
96	CXCR4 (fusin)
97	CXCR5 /BLR-1
98	CXCR6
99	D6
100	DAN
101	DANCE
102	DcR3 / TNFRSF6B
103	Decorin
104	Dkk-1
105	Dkk-3
106	Dkk-4
107	DR3 / TNFRSF25
108	DR6 / TNFRSF21
109	Dtk
110	EDA-A2
111	EDAR
112	EDG-1
113	EGF
114	EGF R / ErbB1
115	EG-VEGF / PK1
116	EMAP-II
117	ENA-78
118	Endocan
119	Endoglin / CD105
120	Endostatin
121	EN-RAGE
122	Eotaxin / CCL11
123	Eotaxin-2 / MPIF-2
124	Eotaxin-3 / CCL26
125	Epiregulin
126	ErbB2
127	ErbB3
128	ErbB4
129	Erythropoietin
130	E-Selectin
131	ETL
132	FADD
133	FAM3B
134	Fas / TNFRSF6
135	Fas Ligand
136	FGF Basic
137	FGF-BP
138	FGF R3
139	FGF R4
140	FGF R5
141	FGF-4
142	FGF-5
143	FGF-6
144	FGF-7 / KGF
145	FGF-8
146	FGF-9
147	FGF-10 / KGF-2
148	FGF-11
149	FGF-12
150	FGF-13 1B
151	FGF-16
152	FGF-17
153	FGF-18
154	FGF-19
155	FGF-20
156	FGF-21
157	FGF-23
158	FLRG
159	Flt-3 Ligand
160	Follistatin
161	Follistatin-like 1
162	Fractalkine
163	Frizzled-1
164	Frizzled-3
165	Frizzled-4
166	Frizzled-5
167	Frizzled-6
168	Frizzled-7
169	Galectin-3
170	GASP-1 /
171	GASP-2 / WFIKKN
172	GCP-2 / CXCL6
173	GCSF
174	G-CSF R / CD 114
175	GDF1
176	GDF3
177	GDF5
178	GDF8
179	GDF9
180	GDF11
181	GDF-15
182	GDNF
183	GFR alpha-1
184	GFR alpha-2
185	GFR alpha-3
186	GFR alpha-4
187	GITR / TNFRF18
188	GITR Ligand / TNFSF18
189	Glucagon
190	Glut1
191	Glut2
192	Glut3
193	Glut5
194	Glypican 3
195	Glypican 5
196	GM-CSF
197	GM-CSF R alpha
198	Granzyme A
199	GREMLIN
200	GRO
201	GRO-a
202	Growth Hormone (GH)
203	Growth Hormone R (GHR)
204	HB-EGF
205	HCC-4 / CCL16
206	HCR / CRAM-A/B
207	Hepassocin
208	Heregulin / NDF / GGF /
209	HGF
210	HGFR
211	HRG-alpha
212	HRG-beta 1
213	HVEM / TNFRSF14
214	I-309
215	ICAM-1
216	ICAM-2
217	ICAM-3 (CD50)
218	ICAM-5
219	IFN-alpha / beta R1
220	IFN-alpha / beta R2
221	IFN-beta
222	IFN-gamma
223	IFN-gamma R1
224	IGFBP-1
225	IGFBP-2
226	IGFBP-3
227	IGFBP-4
228	IGFBP-6
229	IGFBP-rp1 / IGFBP-7
230	IGF-I
231	IGF-I SR
232	IGF-II
233	IGF-II R
234	IL-1 alpha
235	IL-1 beta
236	IL-1 F5 / FIL1delta
237	IL-1 F6 / FIL1 epsilon
238	IL-1 F7 / FIL1 zeta
239	IL-1 F8 / FIL1 eta
240	IL-1 F9 / IL-1 H1
241	IL-1 F10 / IL-1HY2
242	IL-1 R3 / IL-1 R AcP
243	IL-1 R4 /ST2
244	IL-1 R6 / IL-1 Rrp2
245	IL-1 R8
246	IL-1 R9
247	IL-1 ra
248	IL-1 sRI
249	IL-1 sRII
250	IL-2
251	IL-2 R alpha
252	IL-2 R beta /CD122
253	IL-2 R gamma
254	IL-3
255	IL-3 R alpha
256	IL-4
257	IL-4 R
258	IL-5
259	IL-5 R alpha
260	IL-6
261	IL-6 R
262	IL-7
263	IL-7 R alpha
264	IL-8
265	IL-9
266	IL-10
267	IL-10 R alpha
268	IL-10 R beta
269	IL-11
270	IL-12 p40
271	Blank
272	Blank
273	Blank
274	Blank
275	IL-12 p70
276	IL-12 R beta 1
277	IL-12 R beta 2
278	IL-13
279	IL-13 R alpha 1
280	IL-13 R alpha 2
281	IL-15
282	IL-15 R alpha
283	IL-16
284	IL-17
285	IL-17B
286	IL-17B R
287	IL-17C
288	IL-17D
289	IL-17E
290	IL-17F
291	IL-17R
292	IL-17RC
293	IL-17RD
294	IL-18 BPa
295	IL-18 R alpha /IL-1 R5
296	IL-18 R beta /AcPL
297	IL-19
298	IL-20
299	IL-20 R alpha
300	IL-20 R beta

RayBio® Biotin Label-based Human Antibody List...continued

301 Internal Control 1a
302 Internal Control 1b
303 Internal Control 1c
304 Blank
305 IL-21
306 IL-21 R
307 IL-22
308 IL-22 BP
309 IL-22 R
310 IL-23
311 IL-23 R
312 IL-24
313 IL-26
314 IL-27
315 IL-28A
316 IL-29
317 IL-31
318 IL-31 RA
319 Inhibin A
320 Inhibin B
321 Insulin
322 Insulin R
323 Insulysin / IDE
324 IP-10
325 I-TAC / CXCL11
326 Kininostatin / kininogen
327 Kremen-1
328 Kremen-2
329 Lck
330 Latent TGF-beta bp1
331 Blank
332 Blank
333 Blank
334 Blank
335 LBP
336 LECT2
337 Lefty - A
338 Leptin R
339 Leptin (OB)
340 LFA-1 alpha
341 LIF
342 LIF R alpha
343 LIGHT / TNFSF14
344 Lipocalin-1
345 LRP-1
346 LRP-6
347 L-Selectin (CD62L)
348 Luciferase
349 Lymphotactin / XCL1
350 Lymphotoxin beta / TNFSF3
351 Lymphotoxin beta R / TNFRSF3
352 MAC-1
353 MCP-1
354 MCP-2
355 MCP-3
356 MCP-4 / CCL13
357 M-CSF
358 M-CSF R
359 MDC
360 MFG-E8
361 MFRP
362 MIF
363 MIG
364 MIP-1a
365 MIP-1b
366 MIP-1d
367 MIP 2
368 MIP-3 alpha
369 MIP-3 beta
370 MMP-1
371 MMP-2
372 MMP-3
373 MMP-7
374 MMP-8
375 MMP-9
376 MMP-10
377 MMP-11 /Stromelysin-3
378 MMP-12
379 MMP-13
380 MMP-14
381 MMP-15
382 MMP-16 / MT3-MMP
383 MMP-19
384 MMP-20
385 MMP-24 / MT5-MMP
386 MMP-25 / MT6-MMP
387 Musk
388 MSP alpha Chain
389 MSP beta-chain
390 NAP-2
391 NCAM-1 / CD56
392 Neuritin
393 NeuroD1
394 Neuropilin-2
395 Neurturin
396 NGF R
397 NOV / CCN3
398 NRG1 Isoform GGF2
399 NRG1-alpha / HRG1-alpha
400 NRG1-beta1 / HRG1-beta1
401 NRG2
402 NRG3
403 NT-3
404 NT-4
405 Orexin A
406 Orexin B
407 OSM
408 Osteoactivin / GPNMB
409 Osteocrin
410 Osteoprotegerin / TNFRSF11B
411 OX40 Ligand / TNFSF4
412 PARC / CCL18
413 PD-ECGF
414 PDGF R alpha
415 PDGF R beta
416 PDGF-AA
417 PDGF-AB
418 PDGF-BB
419 PDGF-C
420 PDGF-D
421 PECAM-1 /CD31
422 Pentraxin3 / TSG-14
423 Persephin
424 PF4 / CXCL4
425 PlGF
426 PLUNC
427 Pref-1
428 Progranulin
429 Prolactin
430 P-selectin
431 RAGE
432 RANK / TNFRSF11A
433 RANTES
434 RELM beta
435 RELT / TNFRSF19L
436 ROBO4
437 S100 A8/A9
438 S100A10
439 SAA
440 SCF
441 SCF R /CD117
442 SDF-1 / CXCL12
443 sFRP-1
444 sFRP-3
445 sFRP-4
446 sgp130
447 SIGIRR
448 Siglec-5/CD170
449 Siglec-9
450 SLPI
451 Smad 1
452 Smad 4
453 Smad 5
454 Smad 7
455 Smad 8
456 SMDF / NRG1Isoform
457 Soggy-1
458 Sonic Hedgehog (Shh N-terminal)
459 SPARC
460 Spinesin
461 TACI / TNFRSF13B
462 Tarc
463 TCCR / WSX-1
464 TECK / CCL25
465 TFPI
466 TGF-alpha
467 TGF-beta 1
468 TGF-beta 2
469 TGF-beta 3
470 TGF-beta 5
471 TGF-beta RI / ALK-5
472 TGF-beta RII
473 TGF-beta RIIb
474 TGF-beta RIII
475 Thrombopoietin (TPO)
476 Thrombospondin (TSP)
477 Thrombospondin-1
478 Thrombospondin-2
479 Thrombospondin-4
480 Thymopoietin
481 Blank
482 Blank
483 Blank
484 Tie-1
485 Tie-2
486 TIMP-1
487 TIMP-2
488 TIMP-3
489 TIMP-4
490 TL1A / TNFSF15
491 TLR1
492 TLR2
493 TLR3
494 TLR4
495 TMEFF1 / Tomoregulin-1
496 TMEFF2
497 TNF-alpha
498 TNF-beta
499 TNF RI / TNFRSF1A
500 TNF RII / TNFRSF1B
501 TRADD
502 TRAIL / TNFSF10
503 TRAIL R1 / DR4 / TNFRSF10A
504 TRAIL R2 / DR5 / TNFRSF10B
505 TRAIL R3 / TNFRSF10C
506 TRAIL R4 / TNFRSF10D
507 TRANCE
508 Neg
509 Neg
510 Neg
511 Positive 2a
512 Positive 2b
513 Positive 2c
514 Blank
515 TREM-1
516 TROY / TNFRSF19
517 TSG-6
518 TSLP
519 TWEAK / TNFSF12
520 TWEAK R / TNFRSF12
521 Ubiquitin+1
522 uPA
523 uPAR
524 Vasorin
525 VCAM-1 (CD106)
526 VE-Cadherin
527 VEGF
528 VEGF R2 (KDR)
529 VEGF R3
530 VEGF-B
531 VEGF-C
532 VEGF-D
533 VEGI / TNFSF15
534 WIF-1
535 WISP-1 / CCN4
536 XEDAR
537 Neg
538 Internal Control 2a
539 Internal Control 2b
540 Internal Control 2c

VII. 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	Don't make too low dilution Or concentrate sample
	Improper storage of kit	Store kit at suggested temperature
High background	Sample is too concentrated	Use more diluted sample
	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

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