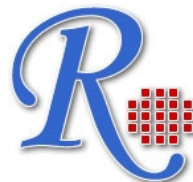


RayBio[®] Biotin Label-based Human Antibody Array I

**For the Simultaneous Detection of the Expression Levels of
507 Human Proteins in Cell Culture Supernates.**

**User Manual
(Revised Aug 19, 2011)**

**(Cat#: AAH-BLM-1-2;
AAH-BLM-1-4)**

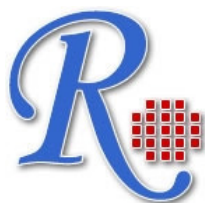


RayBiotech, Inc.

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

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RayBiotech, Inc

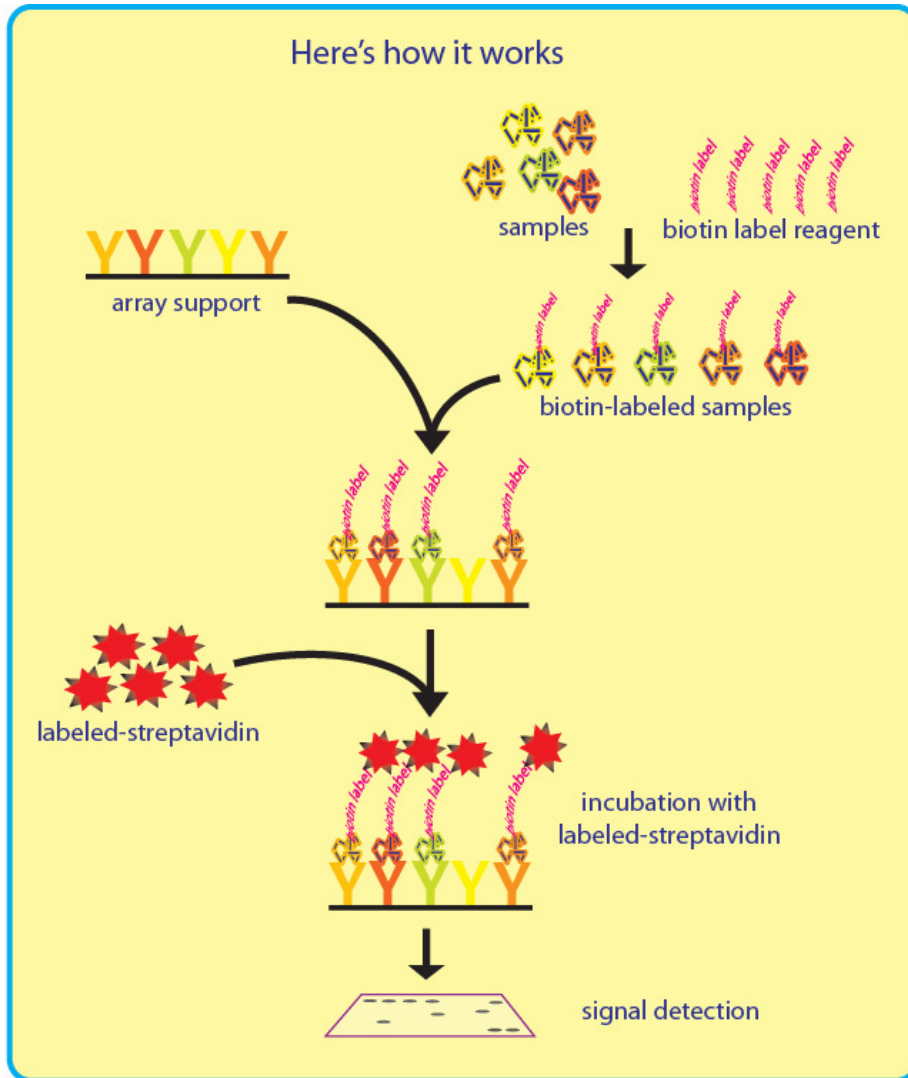
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I. Introduction

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, soluble adhesion molecules and other proteins in cell culture supernate and serum. 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. The biotin-labeled sample is then added onto array membrane and incubated at room temperature. After incubation with HRP-streptavidin, the signals can be visualized by chemiluminescence.



II. Materials Provided

Upon receipt, the Box 1 should be stored at -20°C and Box 2 should be stored at 4°C . Please use within 6 months from the date of shipment. After initial use, the Blocking Buffer, Stop solution, HRP-Conjugated Streptavidin, Detection Buffer C and D should be stored

at 4 °C to avoid repeated freeze-thaw cycles. The Array Membrane and Internal Control should be kept at -20 °C.

Box-1 (store at -20 °C):

- Labeling Reagent (Item B, 1 tube for 2 array membranes , and 2 for 4 array membranes)
- Internal control (Item C, 1 tube for 2 array membranes , and 2 for 4 array membranes)
- Stop Solution (Item D, 50 µl)
- RayBio® Biotin label-based human antibody array I (Item E, 2 for Cat#: AAH-BLM-1-2, and 4 for Cat#: AAH-BLM-1-4)
- Blocking Buffer (Item F, 30 ml for each bottle, 2 bottles for 2 array membranes , and 4 for 4 array membranes)
- 500X HRP-Conjugated Streptavidin Concentrate (Item I, 100 µl)
- Detection Buffer C (Item K, 5 ml for 2 membranes, and 10 ml for 4 membranes)
- Detection Buffer D (Item L, 5 ml for 2 membranes, and 10 ml for 4 membranes)
- Plastic sheet

Box 2 (store at 4 °C):

- Dialysis tube and Floating Rack (Item A, 2 tubes for 2 array membranes, and 4 for 4 array membranes)
- 20X Wash Buffer I (Item G, 30ml)
- 20X Wash Buffer II (Item H, 30ml)
- Spin Column (Item J, 2 columns for 2 array membranes, and 4 for 4 array membranes)
- Plate (2 plates for 2 array membranes, and 4 for 4 array membranes)

III. Additional Materials Required

- 1X PBS, pH=8.0
- Shaker
- 2~5 ml tube
- 50 ml conical collection tube
- Distilled water
- Kodak X-Omat™ AR film (REF 165 1454) and film processor or Chemiluminescence imaging system

IV. Overview and General Considerations

A. Handling Array Membranes

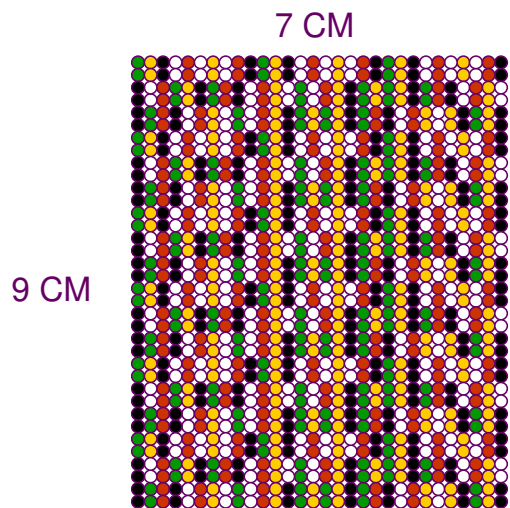
- Always use forceps to handle membranes, and grip the membranes by the edges only.
- Never allow array membranes to dry during experiments.
- Avoid touch Array membrane by hand, tips or any sharp tools.

B. Incubation

- Completely cover membranes with sample or buffer during incubation, and cover eight-well tray with lid to avoid drying.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Several incubation steps such as step 3 in page 10 (sample incubation) or step 7 in page 11 (HRP-streptavidin incubation) may be done at 4 °C for overnight.

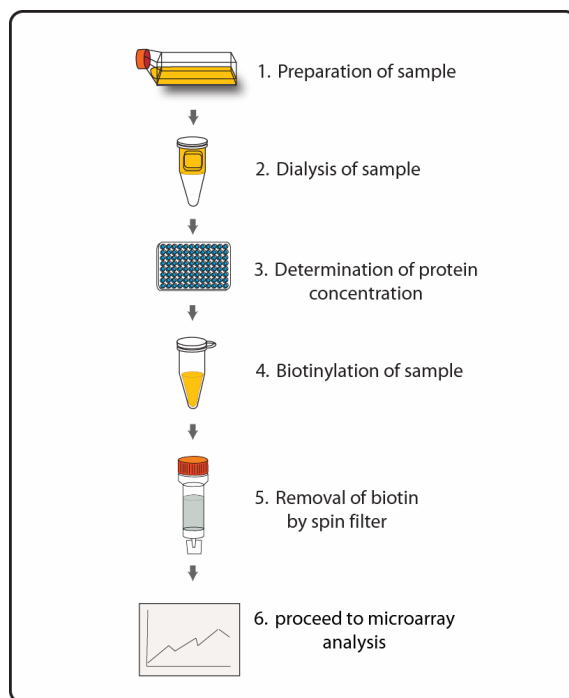
V. Protocol

Layout of Array Membrane



30 columns x 36 rows

Assay Diagram



A. Preparation of Samples

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 1×10^6 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, aliquoted and stored at $-80\text{ }^{\circ}\text{C}$ until use. Meanwhile, the cells are also collected and the total protein concentration is determined. For each sample it is recommended that the concentration of the supernates and cell lysate (help normalize different cell culture supernates) be 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.*

*** Culture times may vary depending on your cell lines and
research.*

B. Dialysis of Sample

The cell culture supernates should be dialyzed with a Dialysis tube (Item A) before the biotin-labeling procedure. We recommend loading 2.5~3.0 ml cell culture supernates into a dialyzer and dialyzing with at least 2,000 ml 1X PBS buffer (pH = 8) at $4\text{ }^{\circ}\text{C}$. 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, 1.0 g KCl, 40 g NaCl, 1.0 g KH_2PO_4 , 5.75 g Na_2HPO_4 dissolve in 4,500 ml deionized or distilled water. Adjust pH=8.0 with 1M NaOH and adjust final volume to 5,000 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 μ l 1X PBS, pH=8.0 into the Internal Control tube, pipette up and down to dissolve the powder. Transfer 2 ml dialyzed sample into a new tube. Add 40 μ l prepared Internal Control into the tube. Mix well.
2. Immediately before use, briefly spin down the Labeling Reagent tube (Item B). Add 100 μ l 1X PBS into the tube, pipette up and down or vortex 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 for 30 min with gentle shaking. Gently tap the tube to mix the reaction solution every 5 min.

- * *7.2 μ l of 1X Labeling Reagent for labeling 1 mg total protein in 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.

4. Add 5 μ l Stop Solution into the above reaction solution and then use the spin column to remove free biotin.
 - a). Twist off the spin column's bottom closure and loosen the cap. Place the column into a 50 ml collection tube.
 - b). Centrifuge column at 1,000 g for 3 minutes to remove storage solution.

Note: The resin will appear compacted after centrifugation.

- c). Add 5 ml 1X PBS into column, centrifuge at 1,000 g for 3 minutes to remove 1X PBS. Repeat additional 2 times to wash the column.
- d). Place the column in a new collection tube, slowly load the sample to the center of the compact resin bed.
- e). Centrifuge the column at 1,000 g for 3 minutes to collect sample. Stored at -80°C until testing. Discard column after use.

D. Blocking and Incubation

1. Place each membrane into the provided tray (“-” mark is on the antibody printed side).

Note: The printed side should be facing upward.

2. Add 8 ml Blocking Buffer and incubate at room temperature with gentle shaking for 1 hour to block membranes.
3. Decant Blocking Buffer from each container. Add 8 ml of sample into each array membrane, and cover with the lid. Incubate at room temperature with gentle shaking for 2 hours. Dilute sample using Blocking Buffer.

Note: 1). We recommended using 8 ml of 5-fold diluted cell culture supernates which have been biotin-labeled. Dilute sample using Blocking Buffer.

Note: 2). The amount of sample used depends on the abundance of protein. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further.

Note: 3). Incubation may be done at room temperature for 2 hours. Over night at 4°C

4. Decant the samples from each container, and wash 3 times with 20 ml of 1X Wash Buffer I at room temperature with shaking. 5

min per wash. Dilute 20X Wash Buffer I with deionized or distilled water.

5. Decant the 1X Wash Buffer I from each container. Wash 3 times with 20 ml of 1X Wash Buffer II at room temperature.
6. Decant the 1X Wash Buffer II. Add 8 ml of 500 fold diluted HRP-conjugated streptavidin (e.g. add 36 μ l of HRP-conjugated streptavidin to 18 ml of Blocking Buffer) to each membrane.

Note: Mix tube containing 500X HRP-Conjugated Streptavidin well before use since precipitation may form during storage.

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

Note: incubation may be done at 4⁰C for overnight.

8. Wash as directed in steps 4 and 5.

E. Detection

*** Do not let the membrane dry out during detection. The detection process must be completed within 40 minutes without stopping.**

1. Add 4.2 ml of Detection Buffer C and 4.2 ml of Detection Buffer D into a tube (for detecting 2 membranes); Mix both solutions; Drain off excess wash buffer. Place membrane protein side up (“-” mark is on the protein side top left corner) on a clean plastic plate or its cover (provided in the kit). Pipette 4ml of the mixed

Detection Buffer on to each membrane and incubate at room temperature for 2 minutes with shaking. Ensure that the detection mixture is evenly covering the membrane without any air bubbles.

2. Gently place the membrane with forceps, protein side up, on a piece of plastic sheet (“-” mark is on the protein side top left corner). Cover the array with another piece of plastic sheet. Gently smooth out any air bubbles. Avoid using pressure on the membrane. Work as quickly as possible.
3. The signal can be detected directly from the membrane using a chemiluminescence imaging system or by exposing the array to x-ray film (we recommend using Kodak X-Omat™ AR film) with subsequent development. Expose the membranes for 40 Seconds. Then re-expose the film according to the intensity of signals. If the signals are too strong (background too high), reduce exposure time (eg, 5–30 seconds). If the signals are too weak, increase exposure time (eg, 5–20 min or overnight). Or re-incubate membranes overnight with 1X HRP-conjugated streptavidin, and repeat detection on the second day.
4. Save membranes at –20 °C to –80 °C for future reference.

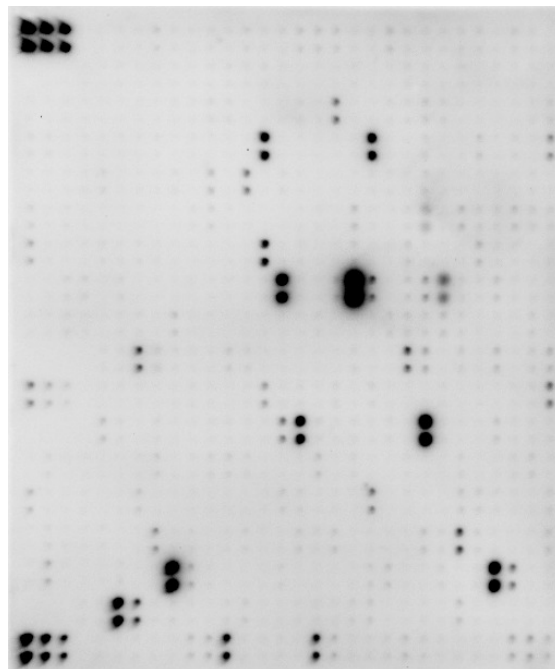
VI. Interpretation of Results:

The following figure shows the RayBio® **Biotin-label-based Array I** probed with cell culture supernates. The image was captured using a chemiluminescence imaging system. One important parameter is the background signal. To obtain the best

results, we suggest that several exposures be attempted. By comparing the signal intensities, relative expression levels of target proteins can be made. The intensities of signals can be quantified by densitometry. 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 to its target varies significantly between antibodies. The intensity detected on the array with each antibody depends on this 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.



RayBio® Biotin Label-based Human Antibody Array I Map –
 Larger versions of this can obtained by contacting technical
 support at 770-729-2992.

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	
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	
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	
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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B	B	B	B	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	
B	B	B	B	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	
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	
B	B	B	B	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	
B	B	B	B	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	
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B	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	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	

RayBio® Biotin Label-based Human Antibody List

1 Positive 1a	61 CCR4	121 EN-RAGE	181 GDF-15	241 IL-1 F10 / IL-1HY2
2 Positive 1b	62 CCR5	122 Eotaxin / CCL11	182 GDNF	242 IL-1 R3 / IL-1 R AcP
3 Positive 1c	63 CCR6	123 Eotaxin-2 / MPIF-2	183 GFR alpha-1	243 IL-1 R4 /ST2
4 neg	64 CCR7	124 Eotaxin-3 / CCL26	184 GFR alpha-2	244 IL-1 R6 / IL-1 Rrp2
5 6Ckine	65 CCR8	125 Epregrulin	185 GFR alpha-3	245 IL-1 R8
6 Activin A	66 CCR9	126 ErbB2	186 GFR alpha-4	246 IL-1 R9
7 Activin B	67 CD14	127 ErbB3	187 GTR / TNFRF18	247 IL-1 ra
8 Activin C	68 CD27 / TNFRSF7	128 ErbB4	188 GTR Ligand / TNFSF18	248 IL-1 sRI
9 Activin RIA / ALK-2	69 CD30 / TNFRSF8	129 Erythropoietin	189 Glucagon	249 IL-1 sRII
10 Activin RIB / ALK-4	70 CD30 Ligand / TNFSF8	130 E-Selectin	190 Glut1	250 IL-2
11 Activin RII A/B	71 CD40 / TNFRSF5	131 Endothelin	191 Glut2	251 IL-2 R alpha
12 Activin RIIA	72 CD40 Ligand / TNFSF5 /CD154	132 FADD	192 Glut3	252 IL-2 R beta /CD122
13 Adiponectin / Acrp30	73 CD 163	133 FAM3B	193 Glut5	253 IL-2 R gamma
14 AgRP	74 Cerberus 1	134 Fas / TNFRSF6	194 Glypican 3	254 IL-3
15 ALCAM	75 Chem R23	135 Fas Ligand	195 Glypican 5	255 IL-3 R alpha
16 Angiogenin	76 Chordin-Like 1	136 FGF Basic	196 GM-CSF	256 IL-4
17 Angiopoietin-1	77 Chordin-Like 2	137 FGF-BP	197 GM-CSF R alpha	257 IL-4 R
18 Angiopoietin-2	78 Csk	138 FGF R3	198 Granzyme A	258 IL-5
19 Angiopoietin-4	79 CLC	139 FGF R4	199 GREMLIN	259 IL-5 R alpha
20 Angiopoietin-like 1	80 CNTF	140 FGF R5	200 GRO	260 IL-6
21 Angiopoietin-like 2	81 CNTF R alpha	141 FGF-4	201 GRO-a	261 IL-6 R
22 Angiopoietin-like Factor	82 Coagulation Factor III / Tissue Factor	142 FGF-5	202 Growth Hormone (GH)	262 IL-7
23 Angiostatin	83 CRIM 1	143 FGF-6	203 Growth Hormone R (GHR)	263 IL-7 R alpha
24 APJ	84 Cripto-1	144 FGF-7 / KGF	204 HB-EGF	264 IL-8
25 AR (Amphiregulin)	85 CRTH-2	145 FGF-8	205 HCC-4 / CCL16	265 IL-9
26 APRIL	86 Cryptic	146 FGF-9	206 HCR / CRAM-A/B	266 IL-10
27 Artemin	87 CTACK / CCL27	147 FGF-10 / KGF-2	207 Hepassocin	267 IL-10 R alpha
28 Axl	88 CTGF / CCN2	148 FGF-11	208 Heregulin / NDF / GGF / Neuregulin	268 IL-10 R beta
29 B7-1 /CD80	89 CTLA-4 /CD152	149 FGF-12	209 HGF	269 IL-11
30 BAFF R / TNFRSF13C	90 CV-2 / Crossveinless-2	150 FGF-13 1B	210 HGFR	270 IL-12 p40
31 neg	91 CXCL14 / BRAK	151 FGF-16	211 HRG1-alpha / NRG1-alpha	271 Blank
32 neg	92 CXCL16	152 FGF-17	212 HRG1-beta1 / NRG1-beta1	272 Blank
33 neg	93 CXCR1 / IL-8 RA	153 FGF-18	213 HVEM / TNFRSF14	273 Blank
34 BCMA / TNFRSF17	94 CXCR2 / IL-8 RB	154 FGF-19	214 I-309	274 Blank
35 BD-1	95 CXCR3	155 FGF-20	215 ICAM-1	275 IL-12 p70
36 BDNF	96 CXCR4 (fusin)	156 FGF-21	216 ICAM-2	276 IL-12 R beta 1
37 beta-Catenin	97 CXCR5 /BLR-1	157 FGF-23	217 ICAM-3 (CD50)	277 IL-12 R beta 2
38 beta-Defensin 2	98 CXCR6	158 FLRG	218 ICAM-5	278 IL-13
39 beta-NGF	99 D6	159 Flt-3 Ligand	219 IFN-alpha / beta R1	279 IL-13 R alpha 1
40 BIK	100 DAN	160 Follistatin	220 IFN-alpha / beta R2	280 IL-13 R alpha 2
41 BLC / BCA-1 / CXCL13	101 DANCE	161 Follistatin-like 1	221 IFN-beta	281 IL-15
42 BMP-2	102 Dcr3 / TNFRSF6B	162 Fractalkine	222 IFN-gamma	282 IL-15 R alpha
43 BMP-3	103 Decorin	163 Frizzled-1	223 IFN-gamma R1	283 IL-16
44 BMP-3b / GDF-10	104 Dkk-1	164 Frizzled-3	224 IGFBP-1	284 IL-17
45 BMP-4	105 Dkk-3	165 Frizzled-4	225 IGFBP-2	285 IL-17B
46 BMP-5	106 Dkk-4	166 Frizzled-5	226 IGFBP-3	286 IL-17B R
47 BMP-6	107 DR3 / TNFRSF25	167 Frizzled-6	227 IGFBP-4	287 IL-17C
48 BMP-7	108 DR6 / TNFRSF21	168 Frizzled-7	228 IGFBP-6	288 IL-17D
49 BMP-8	109 Dtk	169 Galectin-3	229 IGFBP-rp1 / IGFBP-7	289 IL-17E
50 BMP-15	110 EDA-A2	170 GASP-1 / WFIKKNRP	230 IGF-I	290 IL-17F
51 BMPR-IA / ALK-3	111 EDAR	171 GASP-2 / WFIKKN	231 IGF-I SR	291 IL-17R
52 BMPR-IB / ALK-6	112 EDG-1	172 GCP-2 / CXCL6	232 IGF-II	292 IL-17RC
53 BMPR-II	113 EGF	173 GCSF	233 IGF-II R	293 IL-17RD
54 BTC	114 EGF R / ErbB1	174 G-CSF R / CD 114	234 IL-1 alpha	294 IL-18 BPa
55 Cardiotrophin-1 / CT-1	115 EG-VEGF / PK1	175 GDF1	235 IL-1 beta	295 IL-18 R alpha /IL-1 R5
56 CCL14 / HCC-1 / HCC-3	116 EMAP-II	176 GDF3	236 IL-1 F5 / FIL1delta	296 IL-18 R beta /AcPL
57 CCL28 / VIC	117 ENA-78	177 GDF5	237 IL-1 F6 / FIL1 epsilon	297 IL-19
58 CCR1	118 Endocan	178 GDF8	238 IL-1 F7 / FIL1 zeta	298 IL-20
59 CCR2	119 Endoglin / CD153	179 GDF9	239 IL-1 F8 / FIL1 eta	299 IL-20 R alpha
60 CCR3	120 Endostatin	180 GDF11	240 IL-1 F9 / IL-1 H1	300 IL-20 R beta

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

301 Internal Control 1a	361 MFRP	421 PECAM-1 /CD31	481 Blank
302 Internal Control 1b	362 MIF	422 Pentraxin3 / TSG-14	482 Blank
303 Internal Control 1c	363 MIG	423 Persephin	483 Blank
304 Blank	364 MIP-1a	424 PF4 / CXCL4	484 Tie-1
305 IL-21	365 MIP-1b	425 PIGF	485 Tie-2
306 IL-21 R	366 MIP-1d	426 PLUNC	486 TIMP-1
307 IL-22	367 MIP 2	427 Pref-1	487 TIMP-2
308 IL-22 BP	368 MIP-3 alpha	428 Progranulin	488 TIMP-3
309 IL-22 R	369 MIP-3 beta	429 Prolactin	489 TIMP-4
310 IL-23	370 MMP-1	430 P-selectin	490 TL1A / TNFSF15
311 IL-23 R	371 MMP-2	431 RAGE	491 TLR1
312 IL-24	372 MMP-3	432 RANK / TNFRSF11A	492 TLR2
313 IL-26	373 MMP-7	433 RANTES	493 TLR3
314 IL-27	374 MMP-8	434 RELM beta	494 TLR4
315 IL-28A	375 MMP-9	435 RELT / TNFRSF19L	495 TMEFF1 / Tomoregulin-1
316 IL-29	376 MMP-10	436 ROBO4	496 TMEFF2
317 IL-31	377 MMP-11 /Stromelysin-3	437 S100 A8/A9	497 TNF-alpha
318 IL-31 RA	378 MMP-12	438 S100A10	498 TNF-beta
319 Inhibin A	379 MMP-13	439 SAA	499 TNF RI / TNFRSF1A
320 Inhibin B	380 MMP-14	440 SCF	500 TNF RII / TNFRSF1B
321 Insulin	381 MMP-15	441 SCF R /CD117	501 TRADD
322 Insulin R	382 MMP-16 / MT3-MMP	442 SDF-1 / CXCL12	502 TRAIL / TNFSF10
323 Insulysin / IDE	383 MMP-19	443 sFRP-1	503 TRAIL R1 / DR4 / TNFRSF10A
324 IP-10	384 MMP-20	444 sFRP-3	504 TRAIL R2 / DR5 / TNFRSF10B
325 I-TAC / CXCL11	385 MMP-24 / MT5-MMP	445 sFRP-4	505 TRAIL R3 / TNFRSF10C
326 Kininostatin / kininogen	386 MMP-25 / MT6-MMP	446 sgp130	506 TRAIL R4 / TNFRSF10D
327 Kremen-1	387 Musk	447 SIGIRR	507 TRANCE
328 Kremen-2	388 MSP alpha Chain	448 Siglec-5/CD170	508 Neg
329 Lck	389 MICA	449 Siglec-9	509 Neg
330 Latent TGF-beta bp1	390 NAP-2	450 SLPI	510 Neg
331 Blank	391 NCAM-1 / CD56	451 Smad 1	511 Positive 2a
332 Blank	392 Neuritin	452 Smad 4	512 Positive 2b
333 Blank	393 NeuroD1	453 Smad 5	513 Positive 2c
334 Blank	394 Neuropilin-2	454 Smad 7	514 Blank
335 LBP	395 Neurturin	455 Smad 8	515 TREM-1
336 LECT2	396 NGF R	456 SMDF / NRG1Isoform	516 TROY / TNFRSF19
337 Lefty - A	397 NOV / CCN3	457 Soggy-1	517 TSG-6
338 Leptin R	398 NRG1 Isoform GGF2	458 Sonic Hedgehog (Shh N-terminal)	518 TSLP R
339 Leptin (OB)	399 Nidogen-1	459 SPARC	519 TWEAK / TNFSF12
340 LFA-1 alpha	400 NrCam	460 Spinesin	520 TWEAK R / TNFRSF12
341 LIF	401 NRG2	461 TAC1 / TNFRSF13B	521 Ubiquitin+1
342 LIF R alpha	402 NRG3	462 Tarc	522 uPA
343 LIGHT / TNFSF14	403 NT-3	463 TCCR / WSX-1	523 uPAR
344 Lipocalin-1	404 NT-4	464 TECK / CCL25	524 Vasorin
345 LRP-1	405 Orexin A	465 TFPI	525 VCAM-1 (CD106)
346 LRP-6	406 Orexin B	466 TGF-alpha	526 VE-Cadherin
347 L-Selectin (CD62L)	407 OSM	467 TGF-beta 1	527 VEGF
348 Lipocalin-2	408 Osteoactivin / GPNMB	468 TGF-beta 2	528 VEGF R2 (KDR)
349 Lymphotactin / XCL1	409 Osteocrin	469 TGF-beta 3	529 VEGF R3
350 Lymphotoxin beta / TNFSF3	410 Osteoprotegerin / TNFRSF11B	470 TGF-beta 5	530 VEGF-B
351 Lymphotoxin beta R / TNFRSF3	411 OX40 Ligand / TNFSF4	471 TGF-beta RI / ALK-5	531 VEGF-C
352 MAC-1	412 PARC / CCL18	472 TGF-beta RII	532 VEGF-D
353 MCP-1	413 PD-ECGF	473 TGF-beta RIIB	533 VEGI / TNFSF15
354 MCP-2	414 PDGF R alpha	474 TGF-beta RIII	534 WIF-1
355 MCP-3	415 PDGF R beta	475 Thrombopoietin (TPO)	535 WISP-1 / CCN4
356 MCP-4 / CCL13	416 PDGF-AA	476 Thrombospondin (TSP)	536 XEDAR
357 M-CSF	417 PDGF-AB	477 Thrombospondin-1	537 Neg
358 M-CSF R	418 PDGF-BB	478 Thrombospondin-2	538 Internal Control 2c
359 MDC	419 PDGF-C	479 Thrombospondin-4	539 Internal Control 2b
360 MFG-E8	420 PDGF-D	480 Thymopoietin	540 Internal Control 2a

VII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak signal or no signal	1. Taking too much time for Detection.	1. The whole Detection process must be completed in 30 min.
	2. Film developer does not work properly.	2. Fix film developer.
	3. Did not mix HRP-streptavidin well before use.	3. Mix tube containing HRP-Conjugate Streptavidin well before use since precipitates may form during storage.
	4. Sample is too dilute.	4. Increase sample concentration
	5. Other.	1.Reduce blocking concentration by diluting in 1X Wash Buffer II.
2. Slightly increase HRP concentrations.		
3. Slightly increase biotinylate-antibody concentrations.		
4. Expose film for overnight to detect weak signal.		
Uneven signal	1. Bubbles formed during incubation.	1. Remove bubbles during incubation.
	2. Membranes were not completely covered by solution.	2. Completely cover membranes with solution.
High background	1. Exposure to x-ray file is too long.	1. Decrease exposure time.
	2. Membranes were allowed to dry out during experiment.	2. Completely cover membranes with solution during experiment.
	3. Sample is too concentrated.	3. Use more diluted sample.

VIII. Reference List

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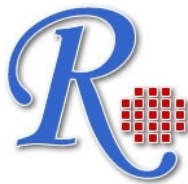
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