RayBio[®] C-Series Human Protein Tyrosine Phosphorylation Antibody Array C1

For the semi-quantitative detection of 507 Tyrosine-phosphorylated human proteins in cell and tissue lysates

Patent Pending Technology

User Manual (Revised July 8th, 2022)

Cat# AAH-PTYR-1-2 (2 Sample Kit) Cat# AAH-PTYR-1-4 (4 Sample Kit)

Please read manual carefully before starting experiment



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C-Series Antibody Arrays

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I. INTRODUCTION

Protein phosphorylation plays an unusually prominent role in cell signaling, development and growth. The RayBio[®] Human Protein Tyrosine Phosphorylation Antibody Array C1 is a very rapid, convenient and sensitive assay to simultaneous detect multiple protein phosphorylations and can be used to monitor activation or function of important biological pathways.

RayBiotech is committed to developing a series of phosphorylation antibody arrays. RayBio[®] Human Protein Tyrosine Phosphorylation Antibody Array C1 is specifically designed for simultaneously identifying the relative levels of Tyrosine phosphorylation of 507 different human proteins in cell lysates. By monitoring the changes in protein tyrosine phosphorylation in your experimental model system, you can verify pathway activation in your cell lines without spending excess time and effort in performing an analysis of immunoprecipitation and/or Western Blot.

By using RayBio[®] Human Protein Tyrosine Phosphorylation Antibody Array C1, treated or untreated cell lysate is added into antibody array membranes. The antibody array membranes are washed and biotinylated anti-phosphotyrosine antibody is used to detect phosphorylated tyrosines on target protein. After incubation with HRP-streptavidin, the signals are visualized by chemiluminescence.

RayBio[®] C-Series Antibody Arrays have several advantages over detection of cytokines using single-target ELISA kits:

- 1. <u>More Data, Same or Less Sample</u>: Antibody arrays provide high-content screening using about the same sample volume as traditional ELISA.
- 2. <u>Global View of Cytokine Expression</u>: Antibody array screening improves the chances for discovering key factors, disease mechanisms, or biomarkers related to cytokine signaling.
- 3. <u>Similar (sometimes better) Sensitivity</u>: As little as 4 pg/ml of MCP-1 can be detected using the C-Series array format. In contrast, our similar MCP-1 ELISA assay has a sensitivity of 40 pg/ml of MCP-1.
- 4. <u>Increased Range of Detection</u>: ELISA assays typically detect a concentration range of 100- to 1000-fold, however, RayBiotech arrays can detect IL-2 at concentrations of 25 to 250,000 pg/ml, a range of 10,000-fold.
- 5. <u>Better Precision</u>: As determined by densitometry, the inter-array Coefficient of Variation (CV) of spot signal intensities is 5-10%, comparing favorably with ELISA testing (CV = 10-15%).

II. HOW IT WORKS

Array support	Samples		
* ****	Incubation of Sample with arrayed antibody supports	}	1-2 hrs
Biotin-Ab	Incubation with Biotiny lated A b	}	1-2 hrs
streptavidin	Incubation with labeled-Streptavidin	}	1 hrs
	Detection of signals		
	Data analysis and graph		

III. COMPONENTS AND STORAGE

Store kit at ≤ -20 °C immediately upon arrival. Kit must use within the 6 months expiration date.

ITEM	COMPONENT	AAH-PTYR-1- 2	AAH-PTYR-1- 4	STORAGE TEMPERATURE AFTER THAWING**						
1	Antibody Arrays	2 membranes	4 membranes	≤-20°C						
2	Blocking Buffer	3 vials (25ml/ea)	5 vials (25ml/ea)	≤-20 C						
3	Biotinylated Anti-Phosphotyrosine Antibody	1 vial	2 vials	2-8°C (for up to 3 days after dilution)						
4	1,000X HRP-Streptavidin Concentrate	1 vial ([50 μl)	2-8 °C						
5	20X Wash Buffer I Concentrate	1 vial (30ml)							
6	20X Wash Buffer II Concentrate	1 vial (30ml)							
7	2X Cell Lysis Buffer Concentrate	1 vial (16 ml)	2-8 °C						
8	Detection Buffer C	1 vial (10 ml)	2 vials (10 ml/ea)	20 C						
9	Detection Buffer D	1 vial (10 ml)	2 vials (10 ml/ea)							
10	Incubation Tray w/ Lid	2 trays	4 trays	Room Temperature						
11	Protease Inhibitor Cocktail 1 vial ≤-20°C									
12	12 Phosphatase Inhibitor Cocktail II 1 vial									
Other	Other Kit Components: Plastic Sheets, Array Map Template, User Manual									

*Each package contains 2 or 4 membranes

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

IV. ADDITIONAL MATERIALS REQUIRED

- Pipettors, pipet tips and other common lab consumables
- Orbital shaker or oscillating rocker
- Tissue paper, blotting paper or chromatography paper
- Adhesive tape or plastic wrap
- Distilled or de-ionized water
- A chemiluminescent blot documentation system:
 - o CCD Camera
 - X-Ray Film and a suitable film processor
 - Gel documentation system
 - o Or another chemiluminescent detection system capable of imaging a western blot

V. SAMPLE TIPS AND GENERAL CONSIDERATIONS

A. Sample Collection, Preparation, and Storage

NOTE: Optimal methods will need to be determined by each experimenter empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.
- Always centrifuge the samples hard after thawing (~10,000 RPM for 2-5 minutes) in order to remove any particulates that could interfere with detection.
- The Cell Lysate can be prepared as follows:
 - For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1500 rpm for 10 min) making sure to remove any remaining PBS before adding Lysis Buffer. Solubilize the cells at 2x10⁷ cells/ml in 1X Lysis Buffer containing Protease Inhibitor Cocktail and Phosphatase Inhibitor Cocktail Set II (see preparation note shown on page 7 under Component Preparation Section). Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 min.

It is recommended that sample protein concentrations be determined using a total protein assay. For incubation with the Phosphorylation Antibody Array I, use at a protein concentration of 50-1000 μ g/ml for cell lysates.

Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.

If you experience high background, you may further dilute your samples. If signals are too weak, the cell lysates can be pretreated by immunoprecipitations before incubation with array membranes. Immunoprecipitations can be done using anti-phosphotyrosine and protein A.

General tips for preparing lysate samples can be viewed on the online Resources page of the website.

B. Sample Types and Recommended Dilutions/Amounts

- **NOTE:** Optimal sample dilutions and amounts will need to be determined by each experimenter empirically but the below recommendations may be used as a starting point. Blocking Buffer (ITEM 2) should be used to dilute samples. Normalize by loading equal amounts of protein per sample.
- **Cell and Tissue Lysates**: load **50 to 1000 μg** of total protein (after at least a 5-fold dilution to minimize the effect of any detergent(s)). Therefore the original lysate concentration should be **250 μg to 5 mg/ml**.

C. Handling Membranes

- The antibody printed side of each membrane is marked by a dash (-) or number (#) in the upper left corner.
- Do not allow membranes to dry out during the experiment or they may become fragile and break OR high and/or uneven background may occur.
- Grasp membranes by the corners or edges only using forceps. DO NOT touch printed antibody spots.

D. Incubations and Washes

- Perform <u>ALL</u> incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/sec) using an orbital shaker or oscillating rocker to ensure complete and even reagent/sample coverage. Rocking/rotating too vigorously may cause foaming or bubbles to appear on the membrane surface which should be avoided.
- All washes and incubations should be performed in the Incubation Tray (ITEM 10) provided in the kit.
- Cover the Incubation Tray with the lid provided during all incubation steps to avoid evaporation and outside debris contamination.
- Ensure the membranes are completely covered with sufficient sample or reagent volume during each incubation.
- Avoid forceful pipetting directly onto the membrane; instead, gently pipette samples and reagents into a corner of each well.
- Aspirate samples and reagents completely after each step by suctioning off excess liquid with a pipette. Tilting the tray so the liquid moves to a corner and then pipetting is an effective method.
- Optional overnight incubations may be performed for the following steps to increase overall spot signal intensities:
 - Sample Incubation
 - Biotinylated Antibody Cocktail Incubation
 - HRP-Streptavidin Incubation
- **NOTE:** Overnight incubations should be performed at 4 °C (also with gentle rocking/shaking). Be aware that longer incubations can also increase the background response so complete liquid removal and washing is critical.

VI. CHEMILUMINESCENCE DETECTION TIPS

- Beginning with adding the detection buffers and ending with exposing the membranes should take no more than 10-15 minutes as the chemiluminescent signals may start to fade at this point.
- Trying multiple exposure times is recommended to obtain optimum results.
- A few seconds to a few minutes is the recommended exposure time range, with 30 seconds to 1 minute being suitable for most samples.

VII. COMPONENT PREPARATION

- **NOTE:** Thaw all reagents to room temperature immediately before use. If wash buffers contain visible crystals, warm to room temperature and mix gently until dissolved.
- **NOTE:** The Biotinylated Antibody Cocktail (ITEM 3) and the HRP-Streptavidin Concentrate (ITEM 4) vials should be briefly centrifuged (~1000 g) before opening to ensure maximum recovery and mixed well as precipitates may form during storage.

ITEM	COMPONENT	PREPARATION	EXAMPLE				
1	Antibody Arrays	No Proparation	N/A				
2	Blocking Buffer	No Preparation	IVA				
3	Biotinylated Antibody Cocktail*	Pipette 1 ml of Blocking Buffer into each vial. Mix gently with a pipette. Transfer the entire contents into a tube containing 11 ml of the Blocking Buffer.	N/A				
4	1,000X HRP-Streptavidin Concentrate	Dilute 1,000-fold with Blocking Buffer. Mix gently with a pipette.	10 μl of 1,000X concentrate + 9990 μl of Blocking Buffer = 10 ml of 1X working solution				
5	20X Wash Buffer I Concentrate	Dilute each 20-fold with distilled or deionized	10 ml of 20X concentrate + 190 ml of water				
6	20X Wash Buffer II Concentrate	water.	=200mlof1X working solution				
7	2X Cell Lysis Buffer Concentrate**	Dilute 2-fold with distilled or deionized water.	10 ml of 2X concentrate + 10 ml of water = 20 ml of 1X working solution				
8	Detection Buffer C						
9	Detection Buffer D	No Preparation	N/A				
10	Incubation Tray w/ Lid						
11	Protease Inhibitor Cocktail	Pipette 60 µl of 1X Cell Lysis Buffer into the vial to prepare 100X Protease Inhibitor Cocktail concentrate.					
12	Phosphatase Inhibitor Cocktail II	Add 180 μl of 1X Lysis Buffer into the vial to prepare 25X Phosphatase Inhibitor Cocktail Set II Concentrate. Dissolve the powder thoroughly by gentle mixing.					

 *1 vial is enough to test 2 membranes

**Only for use for preparing cell or tissue lysates. General tips for preparing lysates and other common sample types can be found on the online Resources Page

Note: Prior to preparing cell or tissue lysates: Add 20 μl Protease Inhibitor Cocktail Concentrate (100X) and 80 μl Phosphatase Inhibitor Cocktail Set II Concentrate (25X) into 1.9 ml 1X Lysis Buffer immediately before use. Mix well.

VIII. PROTOCOL

- **NOTE:** Prepare all reagents and samples immediately prior to use. See Sections V and VII. <u>ALL</u> incubations and washes must be performed under gentle rotation/rocking (~0.5-1 cycle/sec)
- 1) Remove the kit from storage and allow the components to equilibrate to room temperature (RT).
- 2) Carefully remove the Antibody Arrays (ITEM 1) from the plastic packaging and place each membrane (printed side up) into a well of the Incubation Tray (ITEM 10). One membrane per well.

NOTE: The antibody printed side is marked by a dash (-) or number (#) in the upper left corner.

A. Blocking

- 3) Pipette 6 ml of Blocking Buffer (ITEM 2) into each well and incubate for 1 hour at RT.
- 4) Aspirate blocking buffer from each well with a pipette.

B. Sample Incubation

- 5) Pipette 6 ml of diluted or undiluted sample into each well and incubate for 1.5 to 5 hours at RT OR overnight at 4 °C.
- **NOTE:** Longer incubations can help maximize the spot signal intensities. However, doing so can also increase the background response so complete liquid removal and washing is critical.
- 6) Aspirate samples from each well with a pipette.

C. First Wash

- **NOTE:** The 20X Wash Buffer Concentrates I and II (ITEM 5 and 6) must be diluted 20-fold before use. See Section VII for details.
- 7) <u>Wash Buffer I Wash</u>: Pipette 20 ml of **1X** Wash Buffer I into each well and incubate for 5 minutes at RT. Repeat this 2 more times for a total of 3 washes using fresh buffer and aspirating out the buffer completely each time.
- 8) <u>Wash Buffer II Wash</u>: Pipette 20 ml of **1X** Wash Buffer II into each well and incubate for 5 minutes at RT. Repeat this 1 more time for a total of 2 washes using fresh buffer and aspirating out the buffer completely each time.

D. Biotinylated Antibody Cocktail Incubation

NOTE: The Biotinylated Antibody Cocktail (ITEM 3) must be prepared before use. See Section VII for details.

- 9) Pipette 6 ml of the **prepared** Biotinylated Antibody Cocktail into each well and incubate for 1.5 to 2 hours at RT OR overnight at 4°C.
- 10) Aspirate biotinylated antibody cocktail from each well.

E. Second Wash

11) Wash membranes as directed in Steps 7 and 8.

F. HRP-Streptavidin Incubation

- **NOTE:** The 1,000X HRP-Streptavidin Concentrate (ITEM 4) must be diluted before use. See Section VII for details.
- 12) Pipette 6 ml of **1X** HRP-Streptavidin into each well and incubate for 2 hours at RT OR overnight at 4°C.
- 13) Aspirate HRP-Streptavidin from each well.

G. Third Wash

14) Wash membranes as directed in Steps 7 and 8.

H. Chemiluminescence Detection

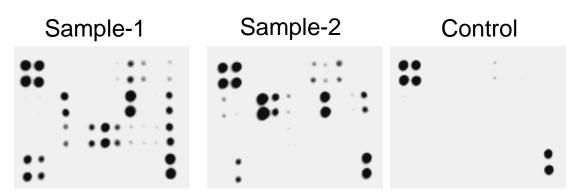
- **NOTE:** *Do not allow membranes to dry out during detection.*
- 15) Transfer the membranes, printed side up, onto a sheet of chromatography paper, tissue paper, or blotting paper lying on a flat surface (such as a benchtop).
- 16) Remove any excess wash buffer by blotting the membrane edges with another piece of paper.
- 17) Transfer and place the membranes, printed side up, onto a plastic sheet (provided) lying on a flat surface.
- **NOTE:** Multiple membranes can be placed next to each other and fit onto a single plastic sheet. Use additional plastics sheets if necessary.
- 18) Into a single clean tube, pipette equal volumes (1:1) of Detection Buffer C (ITEM 8) and Detection Buffer D (ITEM 9). Mix well with a pipette.
- **EXAMPLE**: 4.2 ml of Detection Buffer C + 4.2 ml of Detection Buffer D = 8.4 ml (enough for 2 membrane)
- 19) Gently pipette 4 ml of the Detection Buffer mixture onto each membrane and incubate for 2 minutes at RT (DO NOT ROCK OR SHAKE). <u>Immediately afterwards, proceed to Step 20</u>.
- **NOTE:** Exposure should ideally start within 5 minutes after finishing Step 19 and completed within 10-15 minutes as chemiluminescence signals will fade over time. If necessary, the signals can usually be restored by repeating washing, HRP-Streptavidin and Detection Buffers incubations (Steps 11-19)

- 20) Place another plastic sheet on top of the membranes by starting at one end and gently "rolling" the flexible plastic sheet across the surface to the opposite end to smooth out any air bubbles. The membranes should now be "sandwiched" between two plastic sheets.
- **NOTE:** Avoid "sliding" the top plastic sheet along the membranes' printed surface.
- 21) Transfer the sandwiched membranes to the chemiluminescence imaging system such as a CCD camera (recommended) and expose.
- **NOTE:** Optimal exposure times will vary so performing multiple exposure times is strongly recommended. See Section VI for additional details.

I. <u>Storage</u>

22) To store, without direct pressure, gently sandwich the membranes between 2 plastic sheets (if not already), tape the sheets together or use plastic wrap to secure them, and store at \leq -20 °C for future reference.

IX. TYPICAL RESULTS



Typical results obtained with RayBio[®] C-Series Antibody Arrays

The preceding figures present typical images obtained with RayBio[®] C-Series Antibody Arrays. These membranes were probed with conditioned media from two different cell lines. Membranes were exposed with Kodak X-Omat[®] film at room temperature for 1 minute.

Note the strong signals of the Positive Control spots in the upper left and lower right corners. (See below for further details on the control spots.)

The signal intensity for each antigen-specific antibody spot is proportional to the relative concentration of the antigen in that sample. Comparison of signal intensities for individual antigen-specific antibody spots between and among array images can be used to determine relative differences in expression levels of each analyte sample-to-sample or group-to-group.

X. INTERPRETING THE RESULTS

A. Control Spots

<u>Positive Control Spots (POS)</u> – controlled amount of biotinylated antibody printed onto the array. Used for normalization and to orientate the arrays.

<u>Blank Spots (BLANK)</u> – nothing is printed here. Used to measure the background response.

B. Data Extraction

Visual comparison of array images may be sufficient to see differences in relative protein expression. However, most researchers will want to perform numerical comparisons of the signal intensities (or more precisely, signal *densities*), using 2-D densitometry. Gel/Blot documentation systems and other chemiluminescent or phosphorescent detection systems are usually sold as a package with compatible densitometry software.

Any densitometry software should be sufficient to obtain spot signal densities from your scanned images. One such software program, ImageJ, is available for free from the NIH website along with an array plug-in.

We suggest using the following guidelines when extracting densitometry data from our array images:

- For each array membrane, identify a single exposure that the exhibits a high signal to noise ratio (strong spot signals and low background response). Strong Positive Control Spot signals but not too strong that that they are "bleeding" into one another is ideal. The <u>exposure time does not need to be identical for</u> <u>each array</u>, but Positive Control signals on each array image should have similar intensities.
- Measure the density of each spot using a circle that is roughly the size of one of the largest spots. Be sure to use the <u>same extraction circle</u> dimensions (area, size, and shape) for measuring the signal densities on every array for which you wish to compare the results.
- For each spot, use the <u>summed signal density</u> across the entire circle (ie, total signal density per unit area)

C. Data Analysis

NOTE: RayBiotech offers Microsoft[®] Excel-based Analysis Software Tools for each array kit for automatic analysis. Please visit the website at <u>www.raybiotech.com</u> or contact us for ordering information.

Once the raw numerical densitometry data is extracted, the background must be subtracted and the data normalized to the Positive Control signals to analyze.

<u>Background Subtraction</u>: Select values which you believe best represent the background. If the background is fairly even throughout the membrane, the Negative Control Spots (NEG) and/or Blank Spots (BLANK) should be similar and are accurate for this purpose.

<u>Positive Control Normalization</u>: The amount of biotinylated antibody printed for each Positive Control Spot is consistent from array to array. As such, the intensity of these Positive Control signals can be used to

normalize signal responses for comparison of results across multiple arrays, much like housekeeping genes and proteins are used to normalize results of PCR gels and Western Blots, respectively.

To normalize array data, one array is defined as "Reference Array" to which the other arrays are normalized to. The choice of the Reference Array is arbitrary.

NOTE: The RayBio[®] Analysis Software Tools always designate Array 1/Sample 1 as the Reference Array.

Next, the simple algorithm below can be used to calculate and determine the signal fold expression between like analytes.

X(Ny) = X(y) * P1/P(y)

Where:

P1 = mean signal density of Positive Control spots on reference array P(y) = mean signal density of Positive Control spots on Array "y" X(y) = mean signal density for spot "X" on Array for sample "y" X(Ny)= normalized signal intensity for spot "X" on Array "y"

For example:

Let's determine the relative expression for IL-6 on two different arrays (Arrays 1 and 2). Let's assume that the duplicate signals for the IL-6 spots on each array are identical (or that the signal intensity used in the following calculation is the mean of the two duplicates spots). Also assume the following:

P1 = 2500 P2 = 2700 IL-6 (1) = 300 IL-6 (2) = 455

Then IL-6(N2) = 455 *2500/2700 = 421.30

The fold increase of IL-6(N2) vs IL-6(1) = 421.3/300 = 1.40-fold increase or a 40% increase in the signal intensity of IL-6 in Array 2 vs. Array 1.

XI. 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	POS2	POS3	Blank	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
2	POS1	POS2	POS3	Blank	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
3	Blank	Blank	Blank	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
4	Blank	Blank	Blank	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
5	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
6	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
7	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
8	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
9	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
10	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
11	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173
12	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173
13	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203
14	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203
15	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233
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17	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263
18	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263
19	Blank	Blank	Blank	Blank	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289
20	Blank	Blank	Blank	Blank	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289
21	Blank	Blank	Blank	Blank	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315
22	Blank	Blank	Blank	Blank	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315
23	Blank	Blank	Blank	Blank	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341
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30 31	402	403	404 434	405	406 436	407 437	408	409 439	410 440	411	412	413		415	416	417 447	418	419	420	421 451	422	423	424	425 455	426 456	427	428 458	429 459	430 460	431 461
31	432	433 433	434	435	436	437	438	439	440 440	441	442 442	443	444	445 445	446 446	447	448 448	449 449	450 450	451	452	453 453	454 454	455 455	456	457	458 458	459 459	460	461
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33	Blank	Blank	Blank	462	463	464	465	466	467	400	469	470	471	472	473	474	475	476	477	478	479	480	401	482	403	484	485	Blank	Blank	Blank
35	Blank	Blank	Blank	Blank	403	404	403	400	407	400	409	470	494	472	473	474	473	470	500	501	502	400 503	504	505	506	507	Blank	POS3	POS2	POS1
35	Blank	Blank	Blank	Blank	400	487	400	489	490 490	491	492	493	494 494	495 495	496 496	497	498 498	499	500	501	502	503	504 504	505	506	507	Blank	POS3	POS2	POS1
30	DIATIK	DIATIK	Diarik	DIATIK	400	401	400	409	490	491	492	493	494	490	490	497	490	499	500	501	502	503	504	505	000	507	Diank	P033	r032	F031

POS = Positive Control Spot BLANK = Blank Spot

NOTE: Protein alternative names, accession numbers, and official symbols can be accessed on <u>www.raybiotech.com</u> via the Resources Page.

XII. ARRAY TARGET LIST

18 ANGFU/L 91 CXDEs 164 GGAV2 273 11.1 BB 310 11-14C 388 NHS3 455 HormSpace 19 Angustant 20 AH 10.1 BB 311 Kinnonskant 384 NH3 457 Hyrombageed 21 Arrelinginin 40 DAKLS 156 GCSH 224 LiL1B 314 Letter 385 DTesin A 493 HormBageed 22 Arrelin 50 DEcrin 156 GDF3 241 LiL1B 315 LTEV 388 DSM 461 Thyrmpositet 23 Artain 60 DSA 170 GDF3 241 LiL2 Bata 316 LiftA 310 Detrin 450 ThRP- 24 BDL 102 DBA 172 GDF3 247 LiL3 320 LiftA ThRP- 310 Detrint 456 ThRP- 25 BDA 100 DBA	Number	News	Number	News	Number	Nerre	Ni	News	Ni	News	Number	News	Niccosta	News
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4 Action.0 77 Origin.2 150 166 223 1674 266 16.244 260 MACA 442 Unc. 6 Action.00 79 Crugit. 151 First.ligant 223 16.74. 296 11.75. 171 NACA 444 TTCL 7 CV2 160 Crucit. 151 Intrastitutina 224 11.74. 120 11.72. 121 11.72. NACA 444 TTCL 10 Age0 33 CV2 155 Firstitutina 224 11.17.5 303 11.31.86 376 Mearyline 440 TTFebast 11 Argogenetics.1 64 Crucit. 157 Med.Mol.31.87.7														
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11 Algen 84 CXL 12 Frighted 230 UL127 301 UL127 304 BXCL 127 NoFR 460 167-beak 13 Angegoeite-1 85 CXCL 126 Frighted 222 UL128 305 FrAC 28 NOV 451 ATT2BL 15 Angegoeite-1 88 CXCB 126 Frighted 224 UL128 305 FrAC 452 TG*bask B 15 Angegoeite-1 88 CXCB 124 Hold 124 Hold 324 Hold 452 TG*bask B 16 ANGTL 90 CXCS 162 GAP-2 121 Lid 124 Hold 324 Hold 452 TG*bask B Hold 126 Hold 126 Hold 126 Hold Hold <td< td=""><td>10</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>	10													
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15 Augopotent-4 88 OCCI3 181 Prizided 7 234 FL-LT0 507 Insulation 380 NuCarm 434 GFL-test mit 16 MMGPTL 90 OCCI3 183 GASP-1 226 FL-LR 300 FT-J0 381 MNCar 454 GFL-test mit 18 MMGPTL 91 OCCI3 183 GASP-1 226 FL-LR 331 MIGROL 455 TTF-beta mit 12 Argiotzim 92 DG6 164 GCSP 221 LL-LR 331 MIGROL 455 TTF-deta mit 22 Arrenin 95 DECrin 157 GCSP 224 IL-LR 331 MIGROL 450 Ttropopter 23 MAT 95 DECrin 159 GCSP 224 IL-LR 313 LiceTo-385 044 107 more stappoint 451 Ttropopter 451 Itropopter 453 Ttropopter 451 Ttrop	13	Angiopoietin-1	86	CXCR1	159	Frizzled-5	232	IL-1 F8	305	FACX	378	NOV	451	ATP2B1
16 MACPI11 89 CC/M 16/2 Gaterna 215 HU-HR 808 FI-LO 811 MACC 454 G 072 12 MAGPI17 91 CC/M 164 GAPA 223 H1-HR 811 HI-LK 883 MAC2 455 Thromobaport 13 Argenting 92 DAK 166 GC/P 224 H1-HR 314 H1-KL 885 MT-4 458 Thromobaport 12 Argenting 93 DAK 166 GC/P 224 H1-HR 314 H1-LL 385 MT-4 458 Thromobaport 23 Arterin 95 DC83 169 D41 11/1 <td< td=""><td>14</td><td>Angiopoietin-2</td><td>87</td><td>CXCR2</td><td>160</td><td>Frizzled-6</td><td>233</td><td>IL-1 F9</td><td>306</td><td>Insulin</td><td>379</td><td>GGF2</td><td>452</td><td>TGF-beta RI</td></td<>	14	Angiopoietin-2	87	CXCR2	160	Frizzled-6	233	IL-1 F9	306	Insulin	379	GGF2	452	TGF-beta RI
12 AKGFT2 90 CXG8 163 GGAP-1 216 11.2 R 300 1P-20 312 RNG2 405 TUT-bace MIN 18 Angestatin 92 DKA 165 GCSP 238 11.1 R 318 NT3 457 TUProde Parents 20 AP 93 DKA 165 GCSP 238 11.1 R 318 Kremm-3 385 NT4 458 TUProde Spant 21 APRIK 65 DCS3 163 GGF1 241 11.4 R 318 Lift A 317 Oreain A 400 TUProdeSpant 22 APRIK 69 DK4 172 GGF3 241 11.2 R 118 Lift A 318 Lift A 318 Lift A 316 Lift A 316 Lift A 316 Lift A 317 GGF3 250 Lift A 320 Lift	15	Angiopoietin-4	88	CXCR3	161	Frizzled-7	234	IL-1 F10	307	Insulin R	380	Nidogen-1	453	
18 ANG 14 GGAP2 217 11.1 fb 310 1-14C 888 456 Thrombogued 12 Angulardi 33 DAN 166 GCP2 218 11.1 R8 318 Kincetuni 888 NT-4 457 Thrombogued 21 Angulardi 93 DAN 166 GCS7 210 11.1 R 318 Kreenet. 388 DSM 459 Thrombogued 22 ArRitu 95 DCR3 138 GOP3 241 11.1 R 314 LCR 388 DSM 461 Thrombogued 23 ArLitu 170 GOP3 241 11.2 R 312 Lintu 312 Lintu 314	16	ANGPTL1	89	CXCR4	162	Galectin-3	235	IL-1 R3	308	Insulysin	381	NrCam	454	Grb2
19 Angesterin 22 DE 165 GCSP 238 L1.R8 311 Krinnetten 388 NT-3 497 Hypord Percents 21 Amphringuin 44 Decrin 167 GCSR 240 L1.L7 313 Kremen- 388 NT-4 493 Thrombogond 22 Arternin 66 Decorin 168 GOP1 241 L1.L3 316 L12 389 Orsaccrin 460 Thrombogond 24 Arternin 66 Decorin 169 GOP5 243 L12.B 118 316 GVA 320 Ostescrin 462 Threl-12 25 BOA 100 DB6 174 GOP1 241 L1.3 320 Leptin R 320 Ostescrin 463 ThtM-3 28 BOA 100 DB6 174 GGP1 251 L1.4 320 Leptin R 320 Ostescrin 453 ThtM-3 <	17	ANGPTL2	90	CXCR5	163	GASP-1	236	IL-1 R4	309	IP-10	382	NRG2	455	TGF-beta RIII
D0 APA DAN 166 GCST 239 IL-L8 312 Kreme-1 286 Oresin A 439 Torombogond 22 APRIL 65 Decrin I 66 GGT3 241 IL-L8 314 Lt-B 380 Oresin A 460 Thrombogond 23 Arterin 65 Decrin I 66 GGT3 241 IL-L8 315 TTP2 388 OSM 461 Thrombogond 24 Arterin 6 Decorin I GGT3 241 IL-2 316 LIP 390 Ottosaction 463 Tre-2 25 BO-A 100 DBA 172 GOT3 247 IL-3 310 Liptin 312 UPAL 1914 445 ThM-4 20 BD-T 101 DBA 172 GOT3 247 IL-3 320 UPAL 1914 320 VAGUigand 446 DTM-4 310 DBA 177 GTA 1914-3	18	ANGPTL7	91	CXCR6	164	GASP-2	237	IL-1 R6	310	I-TAC	383	NRG3	456	Thrombopoietin
21 Ampbirguin 4 0 MAC 167 6 GVL 244 11.1 rs 331 LK remen-2 386 Oracin 450 Thrombogond 22 Arternin 60 Decorin 169 GOP3 243 11.1 Rill 335 LK 387 Oracin 400 Thrombogond 24 Auf 97 DWL 170 GOP5 243 11.2 Rill 335 LVT2 390 Obtacorin 462 Tite_1 25 BAFR 9 DWL 170 GOP5 254 11.2 Rill 315 Life/h, 319 391 PAGA 166 Tite/h 28 DOL 100 D66 174 GOP7 143 320 Life/h 393 PAGA 466 Tite/h 28 BLA 175 GON7 248 Lite A lipha 321 Life A lipha 324 Life/h 393 PAGA 470 Tite/h 39 DRAC 105 <td>19</td> <td>Angiostatin</td> <td>92</td> <td>D6</td> <td>165</td> <td>GCP-2</td> <td>238</td> <td>IL-1 R8</td> <td>311</td> <td>Kininostatin</td> <td>384</td> <td>NT-3</td> <td>457</td> <td>Thyroid Peroxidase</td>	19	Angiostatin	92	D6	165	GCP-2	238	IL-1 R8	311	Kininostatin	384	NT-3	457	Thyroid Peroxidase
22 APRIL 95 Deck1 168 GOF1 244 ILI.8 314 Lick 387 Ortenina 460 Thrombognooth 23 Artenin 95 Decrin 130 GOF5 242 ILI.81 315 IECT 380 Oxtescriven 452 Tre-1 25 BPT.1 98 Dik-3 171 GOF5 248 ILI.2 Barla 318 LeftyA. 100 Oxtescriven 452 Tre-2 27 BCAA 100 Diff.3 300 Diff.3 212 GOF5 248 ILI.8 abal LeftyA. 100 Oxtescriven 464 TMM-7 28 BDP1 103 Diff.3 275 GOF8 226 ILI.8 230 Left Alabh 249 FMM-7 230 Left Alabh 240 Left Ala	20	APJ	93	DAN	166	GCSF	239	IL-1 R9	312	Kremen-1	385	NT-4	458	Thrombospondin-1
22 APRIL 95 DerX1 100 GOP3 242 1L:1.8 315 Lix 8 387 Oren in 8 60 Thrombogenet 23 Artsenin 95 BPA-1 98 Dik-3 171 GOP5 242 1L:1.8 315 LEC2 38 Oxtesortim 462 Tre-1 26 BPAF 99 Dik-3 171 GOP5 248 1L/2.8 bran 318 Lefyn. 319 Legin 8 300 Ditsoprotegerin 464 Tite/2 27 BCAM 100 DB8 174 GOP1-15 247 Li-3 320 Legin 8 30 Ditsoprotegerin 464 TitA 28 DDM 107 Dik<175	21	Amphiregulin	94	DANCE	167	G-CSF R	240	IL-1 ra	313	Kremen-2	386	Orexin A	459	Thrombospondin-2
24 Aut 97 Dik-3 170 GOFS 248 Li-2 Baile LiPe Baile Oxteocrition 462 Tre-1 25 BAFR 99 Dik-3 171 GOFS 245 Li-2 Reich 316 Left 2.8 Dittegrothegerin 643 TIMP-2 27 BCAA 100 DB8 173 GOFS 245 Li-2 Reich 318 Left Rein 312 Dixtegrothegerin 644 TIMP-2 28 BOP 101 DB8 174 GOFS 248 Li-8 Reight 3.20 Left Alapht 3.3 BAC 666 TIMP-4 30 Detx Actom 103 EDA 176 GFR alpha-3 230 Li-4 232 Li-5 244 Li-6 246 Li-7 180 POC F-40 70 TUZ 31 Bac 1046 EGF 179 GFR alpha-3 231 Li-5 246 Li-7 230 POC F-40 71 <tur< td=""> TUR <</tur<>	22	APRIL	95	DcR3	168	GDF1	241	IL-1 RI	314	Lck	387	Orexin B	460	Thrombospondin-4
25 87-1 98 Obk-4 17.1 CORB 244 IL-2 alpha 317 LECT 300 Oxteocrin 463 TTR-2 26 APA ID-2 GGMA 100 DB3 172 GOP11 245 IL-2 R parms 310 Leptin 331 Leptin 332 Oxteo Lind 465 TTM-2 28 DD-1 101 DB6 172 GGM1 247 IL-3 320 Leptin 333 PD6CF 466 TTM-3 29 BDT 102 DBK 102 DBK GGM1 104 222 IL-5 234 IL-6 333 PD6F Abc 457 TTR 33 BK 106 EGF 175 GGM1 251 IL-6 125 IL-7 328 IL-2 TTR 110 1108 110 1107 TTR TTR 110 139 PD6F-Ab 471 TTM-4F1 38 BMF-2 1108<	23	Artemin	96	Decorin	169	GDF3	242	IL-1 RII	315	LTBP1	388	OSM	461	Thymopoietin
26 DAF.R 99 DMA-1 172 GOF9 245 II-2.8 hela 318 Letyn A 321 Obsequetation 64.4 TIMP-1 27 GOFM 101 DB6 17.4 GOF-15 247 IL-3 320 Leptin 331 Decorder 65.6 TIMP-3 28 BD1 103 EDAA2 175 GFN alpha-2 250 IL-4 222 UF 333 PDGF # alpha 456 DECGF 677 TIMP-3 31 Baca-MGF 105 EDAA 177 GFR alpha-3 251 IL-5 244 UFA 356 PDGF Med 457 TIMA 32 beta-MGF 106 EGF R 300 GFR alpha-3 251 IL-6 326 UPA-1 358 PDGF Med 471 TIMA 336 BMK-2 108 EMAPI 112 EMAPI 122 EMAPI 123 EVEA 110 DPGF Med 472 TTMF	24	AxI	97	Dkk-1	170	GDF5	243	IL-2	316	LBP	389	Osteoactivin	462	Tie-1
22 BCMA 100 D86 172 GDP11 246 IU-2 332 Leptin 332 Leptin 332 DAGU design 28 BDNF 102 Dts 175 GONF 248 IL-3 321 LEA1 sighs 332 MAC 665 TIMP-4 30 beta-Stermin 103 EDA-42 176 GFR alpha-2 250 IL-4 322 UE ralpha 395 PDGF Ralpha 485 DESAS 31 Bax 106 GGF 177 GFR alpha-2 250 IL-4 322 UE ralpha 396 PDGF-A 471 TU12 33 BtK 106 GGF 177 GFR alpha-2 251 IL-6 326 LIPA-1 399 PDGF-AB 471 TU14 34 BtC-107 GFR alpha-1 252 IL-7 328 LSeglectin 401 PDGF-AB 472 TTMF-1 35 BtMP-4 111 E	25	B7-1	98	Dkk-3	171	GDF8	244	IL-2 R alpha	317	LECT2	390	Osteocrin	463	Tie-2
28 BD.1 101 DB6 174 GDF15 247 11.3 200 Leptin 333 PABC 666 TINH-3 29 DBVF 102 DBK 175 GOF14 248 11.6 314 B33 PABC 666 TINH-3 31 BBX 104 EDAR 177 GFF alpha-4 250 LL4 232 LLF alpha 398 PDGF-AA 470 TLR2 33 BK 106 GGF R 180 GFF alpha-4 251 LL-6 324 LUPcl II 398 PDGF-AA 470 TLR2 34 BLC 106 GGF R 180 GFF alpha-4 252 LL-6 327 LLPG 400 PDGF-BA 470 TLR2 35 BMF-3 108 BAA-HI 112 Endogan 257 LL 331 LPG-L 401 PMGF-BA 470 TMF+3 36 BMF-5 112 Endogan	26	BAFF R	99	Dkk-4	172	GDF9	245	IL-2 R beta	318	Lefty-A	391	Osteoprotegerin	464	TIMP-1
20 BDNF 102 Dtk 175 GDMF 248 IL-3 321 UFA-189 394 PD-CCG 4-67 TIM-4 30 beta-Keim 103 EDM 177 GFR alpha-2 250 IL-4 322 UFR alpha 396 POGF Abpt 468 TIBL 31 BiX 105 EDG-1 178 GFR alpha-3 251 IL-5 324 UIGH1 398 POGF-AA 470 TIBL 33 BiX 106 EOF 179 GFR alpha-3 251 IL-6 325 UIPC-4 90 POGF-AB 471 TIBA 34 BIC-1 107 EDFA 180 GTIT 253 IL-6 326 UIPC-4 90 POGF-AB 472 TIBA 35 BIMP-2 108 EMP-3 108 EMP-3 108 PECM-1 473 TMHF1 36 BMP-3 111 Endogtin 183 Giut-2 2	27	BCMA	100	DR3	173	GDF11	246	IL-2 R gamma	319	Leptin R	392	OX40 Ligand	465	TIMP-2
30 beta-Caterin 103 EDAA 117 GFR alpha-3 250 IL4 332 UF alpha 36 PDOF Alpha 468 DPTAS 31 Bax 104 EDAA 117 GFR alpha-3 251 IL-5 324 UIGHT 397 PDGF-AA 470 TDS2 33 BiK 106 EGF 179 GFR alpha-4 252 IL-5 Ralpha 236 UIGHT 398 PDGF-AB 471 TDB3 34 BIK 106 EGF KeF 181 GITR Ugand 254 IL-6 327 Lipsectin 400 PDGF-AB 472 TDH4 35 BMP-2 108 EGAVEF 181 GITR Ugand 254 IL-6 327 Lipsectin<	28	BD-1	101	DR6	174	GDF-15	247	IL-3	320	Leptin	393	PARC	466	TIMP-3
31 Bax 104 EDA4 177 GFR alpha-2 250 IL-4 321 UF Raiph 360 POGF AAA 470 TUB2 33 BiK 105 EDG-1 178 GFR alpha-4 251 IL-5 324 Ulportalin-1 398 POGF-AA 470 TUB2 34 BiK 105 EGP-FR 180 GTR alpha-4 252 IL-6 325 Ulportalin-1 398 POGF-AB 471 TUB3 35 BMP-2 108 EGAVEGF 180 GTR alpha 22 IL-6 322 ILP-7 328 Leglectin 401 POGF-A 473 TMEFF1 36 BMP-2 108 EMAV-8 115 GIGI3 259 IL-0 332 Ulportalin-2 402 PECM-1 476 TWF-stalpha 33 39 BMP-5 112 Endotatin 186 Glugia 259 IL-10 332 UTB 405 PF4 478 TWF-stalpha	29	BDNF	102	Dtk	175	GDNF	248	IL-3 R alpha	321	LFA-1 alpha	394	PD-ECGF	467	TIMP-4
32 betk-NGF 105 178 GFR alpha-3 251 11-5 244 1107T 377 PDGF-AA 470 TH23 33 BIK 106 EGF R 180 GITR 253 IL5 R alpha 325 Upcrain 397 PDGF-AB 471 TH33 34 BUC 108 EGF R 110 GTR upand 254 IL6 326 ILP-1 399 PDGF-AB 472 TIM4 35 BUM-2 108 EGVEGF 181 GITR upand 254 IL-6 Nather Nather </td <td>30</td> <td>beta-Catenin</td> <td>103</td> <td>EDA-A2</td> <td>176</td> <td>GFR alpha-1</td> <td>249</td> <td>IL-4</td> <td>322</td> <td>LIF</td> <td>395</td> <td>PDGF R alpha</td> <td>468</td> <td>DEFA5</td>	30	beta-Catenin	103	EDA-A2	176	GFR alpha-1	249	IL-4	322	LIF	395	PDGF R alpha	468	DEFA5
33 91K 106 EGF 179 GFR alpha 4 252 IL-5 Alpha 42 Upcrain-1 398 PDGF-AB 471 TUBA 34 BUC 107 EGF R 180 GTR 253 IL-6 326 Lip-1 399 PDGF-AB 471 TUBA 35 BMP-2 100 EMA-78 100 EMA-78 100 EMA-78 TUBA 255 IL-7 322 Lipecin 1.4 400 PDGF-A 474 TMFF1 36 BMP-3 110 EMA-78 118 GUIZ 257 IL-8 330 Lipocain.7 402 PECAM-14 475 TMF-alpha 39 BMP-6 112 Endoglin 185 GUIZ 259 IL-10 332 ITBR 405 PF4 478 TMF BI 41 BMP-6 113 Endoxin 186 Gypican 5 261 IL-10 333 MAC-1 405 Pr4 478 TMAI	31	Bax	104	EDAR	177	GFR alpha-2	250	IL-4 R	323	LIF R alpha	396	PDGF R beta	469	TLR1
34 BLC 107 EFR 130 GTR 233 IL-6 226 128-1 399 PDGF-BB 472 TIMA 35 BMP-2 108 E6-VEGF 181 GTR Ligand 254 IL-6 327 LBP-6 400 PDGF-C 473 TMEF71 36 BMP-3 100 EMAP-11 182 GRR1 255 IL-7 324 L-Selectin 401 PDGF-C 473 TME72 38 BMP-4 111 Endocani 184 Giut2 258 IL-9 333 ITTR 402 PErcAM-1 475 TMF-beta 40 BMP-5 116 Endoritin 186 Giypican 5 261 IL-10 Ribeta 334 MAC-1 406 PIGF-1 480 TRAL 41 BMP-5 115 Eotani 188 Giypican 5 261 IL-12 Ribta 333 MAC-1 407 Pigranulin 482 TRAL 43	32	beta-NGF	105	EDG-1	178	GFR alpha-3	251	IL-5	324	LIGHT	397	PDGF-AA	470	TLR2
35 BMP-2 108 EG-VEGF 181 GITR Ligand 254 IL-6 8 237 BMP-3 109 EMAP-11 182 CBRI 255 IL-7 328 Liselectin 401 PPG-F-0 473 TMEFF1 37 BMP-3b 110 EMA-78 183 Giut1 256 IL-7 Ralpha 329 Lipocalin-2 402 PECAM-1 475 TMF-alpha 38 BMP-4 111 Endocan 184 Giut2 257 IL-8 330 Lipocalin-2 402 PECAM-1 475 TMF-alpha 40 BMP-5 112 Endostatin 188 Giut3 259 IL-10 332 MCP-1 404 PERAPIN 478 TMF Rit 41 BMP-6 115 Edaxin 188 Giycican 3 261 IL-10 335 MCP-1 407 PTML 481 TRAL 43 BMP+15 115 Edaxin 190 GMC-2FF 26	33	BIK	106	EGF	179	GFR alpha-4	252	IL-5 R alpha	325	Lipocalin-1	398	PDGF-AB	471	TLR3
S6 BMP-3 109 EMAP-II 182 CBR1 255 IL-7 328 Lispection 401 PDGF-0 474 TMFF22 37 BMP-3b 110 EMA-78 183 Giut1 256 IL-7 Ralpha 329 Lippectin-2 402 PECAM-1 475 TMF-sipha 38 BMP-4 111 Endostini 184 Giut2 257 IL-8 330 Lippectin-4 403 Petraxin3 476 TMF-sipha 40 BMP-5 113 Endostini 188 Giut2 259 IL-10 332 LTR 405 PF4 478 TNF RI 41 BMP-6 113 Endaxin-2 189 GM-25F 261 IL-12 PA0 334 MCF-1 407 TNF RI TRADD 42 BMPR-15 116 Endaxin-3 190 GM-25F IL-12 PA0 335 MCF-2 408 Pref-1 481 TRALR3 44 BMPR-16	34	BLC	107	EGF R	180	GITR	253	IL-6	326	LRP-1	399	PDGF-BB	472	TLR4
37 BMP-3b 110 EMA-78 184 Glut1 256 IL-7 R alpha 320 Uppoclin-2 402 PECM-11 475 TIMalpha 39 BMP-5 112 Endogin 185 Glut3 257 IL-8 330 Uppoclin-24 402 Percapina 477 TNF RI 40 BMP-6 113 Endostatin 186 Glut3 258 IL-10 332 MAC-1 405 Perceptina 477 TNF RI 41 BMP-7 114 Endostatin 188 Glypican 3 260 IL-10 Rabia 333 MAC-1 405 PFd-4 481 TRAL 42 BMP-81 116 Eotaxin-2 189 GMC-SF 262 IL-11 335 MC-24 408 Pref-1 481 TRAL RA 43 BMPR-16 116 Eotaxin-2 190 GMC-SF 261 IL-12 Ad 336 MC-S-2 411 Presideuin 481 TRAL RA	35	BMP-2	108	EG-VEGF	181	GITR Ligand	254	IL-6 R	327	LRP-6	400	PDGF-C	473	TMEFF1
B BMP-4 111 Endocan 185 Glu3 257 1-8 330 LTB 403 Pentraxin3 476 TM*-beta 39 BMP-5 112 Endostant 185 Glu43 258 IL-9 331 LTB 404 Persphin 477 TM* RI 40 BMP-6 113 Endostant 186 Glu54 259 IL-10 332 LTBR 405 PF4 478 TM* RI 41 BMP-7 114 EN-AGE 187 Glypican 5 261 IL-10 Rajha 333 MAC-1 406 Pref-1 481 TRAIL 43 BMP-15 115 Eotaxin-3 190 GM-CSF 262 IL-11 335 MCP-2 408 Pref-1 481 TRAIL R3 44 BMPR-16 118 Epitrayini 191 GREMUN 263 IL-12 Robta 330 MCSF 411 Prelactin 483 TRAIL R3 45 BMPR-18 <td>36</td> <td>BMP-3</td> <td>109</td> <td>EMAP-II</td> <td>182</td> <td>CBR1</td> <td>255</td> <td>IL-7</td> <td>328</td> <td>L-Selectin</td> <td>401</td> <td>PDGF-D</td> <td>474</td> <td>TMEFF2</td>	36	BMP-3	109	EMAP-II	182	CBR1	255	IL-7	328	L-Selectin	401	PDGF-D	474	TMEFF2
9 BMP-5 112 Endoglin 185 Gluts 258 IL-9 311 TTB 404 Persephin 477 TTNF RII 40 BMP-6 113 Endostatin 186 Gluts 259 IL-10 331 MAC-1 406 PIGF 478 TTNF RII 41 BMP-8 115 Eotaxin 188 Glypican 5 261 IL-10 Rubeta 334 MC-1 406 PIGF 479 TTADD 43 BMP-15 116 Eotaxin-3 190 GM-CSF 262 IL-11 335 MCP-2 408 Pregranulin 481 TTAIL R2 44 BMPR-IA 117 Eotaxin-3 190 GM-CSF Ralpha 263 IL-12 PdO 337 MCP-4 400 Progranulin 482 TTRAL R2 45 BMPR-II 191 GranzmeA 264 IL-12 Rubeta 330 MC-54 411 Pradeta 483 TRAL R2 46 Cardioro	37	BMP-3b	110	ENA-78	183	Glut1	256	IL-7 R alpha	329	Lipocalin-2	402	PECAM-1	475	
40 BMP-6 113 Endostatin 186 Gluts 259 IL-10 332 LTBR 405 PF4 478 TNFRII 41 BMP-7 114 EN-RAGE 187 Glypican 3 260 IL-10 Rajpha 333 MAC-1 406 PIGF 479 TRADD 42 BMP-85 116 Eotaxin-2 189 GM-CSF 262 IL-11 335 MCP-1 407 PLUK 480 TRALL 44 BMP-1A 117 Eotaxin-2 189 GM-CSF 262 IL-12 403 BMCP-3 409 Progranulin 482 TRALL R3 45 BMP-RI 119 ErbR2 192 GREMUN 265 IL-12 Rota1 331 MC-SF 411 P-selectin 483 TRALR R3 46 BMP-RI 112 ErbR4 194 GRO-a 267 IL-13 340 MCSF 411 RANK 486 TRANC1 470	38	BMP-4	111	Endocan	184	Glut2	257	IL-8	330	Lymphotactin	403	Pentraxin3	476	TNF-beta
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XIII. TROUBLESHOOTING GUIDE

PROBLEM	CAUSE	RECOMMENDATION						
	Chemiluminescent imager is not working properly	Contact image manufacturer						
No signals	Too Short Exposure	Expose the membranes longer						
(not even the positive controls spots)	Degradation of components due to improper storage	Store entire kit at ≤ - 20°C. Do not use kit after expiration date. See storage guidelines.						
	Improper preparation or dilution	Centrifuge vial briefly before use, mix well, and do not						
	of the HRP-Streptavidin	dilute more than 1000-fold						
	Waiting too long before exposing	The entire detection process should be completed in 10-15 minutes						
Positive controls spots	Low sample protein levels	Decrease sample dilution, concentrate samples, or load more protein initially						
signals visible but no other	Skipped Sample Incubation Step	Samples must be loaded after the blocking step						
spots	Too Short of Incubations	Ensure the incubations are performed for the appropriate time or try the optional overnight incubation(s)						
	Bubbles present on or below membrane	Don't rock/rotate the tray too vigorously or pipette the sample or reagent with excessive force						
Uneven signals and/or	Insufficient sample or reagent volume	Load enough sample and reagent to completely cover the membrane						
background	Insufficient mixing of reagents	Gently mix all reagents before loading onto the membrane, especially the HRP-Streptavidin and Biotin Antibody Cocktail						
	Rocking/Rotating on an uneven surface while incubating	Rock/rotate on a flat surface or the sample or reagent can "pool" to one side						
	Too much HRP-Streptavidin or Biotinylated Antibody Cocktail	Prepare these signal enhancing components precisely as instructed						
	Membranes dried out	Do not let the membranes dry out during the experiment. Cover the incubation tray with the lid to minimize evaporation						
High background signals or all spots visible	Too High of Sample Protein Concentration	Increase dilution of the sample or load less protein						
	Exposed Too Long	Decrease exposure time						
	Insufficient Washing	Ensure all the wash steps are carried out and the wash buffer is removed completely after each wash step						
	Non-specific binding	Ensure the blocking buffer is stored and used properly.						

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