

RayBio[®] Human Adipokine Antibody Array G series 1

Patent Pending Technology

User Manual (Revised Aug. 25, 2008)

RayBio[®] Human Adipokine Antibody Array G Series 1 (Cat# AAH-ADI-G1-8)

RayBio[®] Human Adipokine Antibody Array Service (Cat# AAH-SER- ADI-G1)

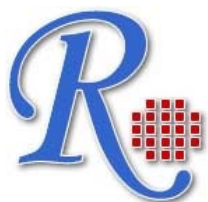
Please read the manual carefully before you start your experiment



RayBiotech, Inc.

**We Provide You With Excellent
Protein Array Systems And Service**

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RayBiotech, Inc.

RayBio[®] Human Adipokine Antibody Array G Series 1 Protocol

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Cytokine Antibody Arrays are RayBiotech patent-pending technology.

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I. Introduction

All cell functions, including cell proliferation, cell death and differentiation, as well as maintenance of health status and development of disease, are controlled by many genes and signaling pathways. New techniques such as cDNA microarrays have enabled us to analyze the global gene expression¹⁻³. However, almost all cell functions are executed by proteins, which cannot be studied by DNA and RNA alone. Experimental analysis clearly shows a disparity between the relative expression levels of mRNA and their corresponding proteins⁴. Therefore, it is critical to analyze the protein profile. Currently, two-dimensional polyacrylamide SDS page coupled with mass spectrometry is the mainstream approach to analyzing multiple protein expression levels^{5,6}. However, the requirement of sophisticated devices and the lack of quantitative measurements greatly limit its broad application. Thus, no simple, cost effective, and rapid method of analysis of multiple protein expression levels has been available to researchers until now.

Our RayBio[®] Human Cytokine Antibody Array is the first commercially available protein array system⁷⁻¹¹. By using the RayBiotech system, scientists can rapidly and accurately identify the expression profiles of multiple cytokines in several hours inexpensively.

The RayBiotech kit (G series) is a glass slide format. The kit provides a highly sensitive approach to simultaneously detect multiple cytokine expression levels from cell culture supernatant, patient's serum, tissue lysate and other sources. The arrays are manufactured using non-contact arrayer. The experimental procedure is simple and can be performed in any laboratory. The signals from G series arrays are detected using a laser scanner.

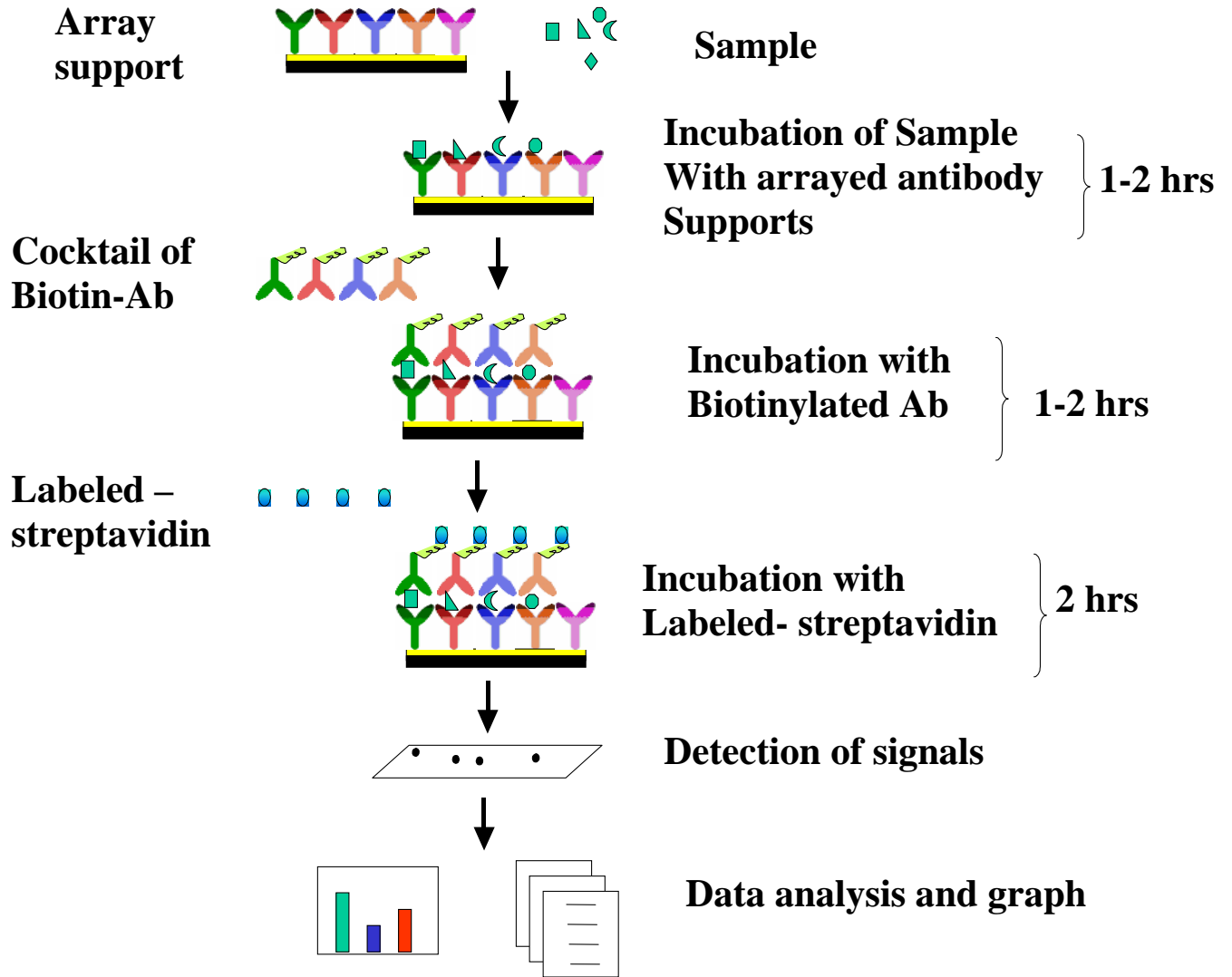
Besides the products listed in this manual, RayBiotech also provides RayBio[®] Human Cytokine Antibody Array G series 1000 for detection of 120 human cytokines in single experiment and RayBio[®] Human Cytokine Antibody Array G series 2000 for detection of 174 human cytokines in single experiment.

Pathway-specific array systems allow investigators to focus on the specific problem and are becoming an increasingly powerful tool in cDNA microarray system. RayBiotech's first protein array system, known as RayBio[®] Human Adipokine Antibody Array, is particularly useful compared with the human adipokine cDNA microarray system. Besides the ability to detect protein expression, RayBiotech's system is a more accurate reflection of active adipokine levels because it only detects secreted adipokines, and no amplification step is needed. Furthermore, it is much simpler, faster, environmentally friendlier, and more sensitive.

Simultaneous detection of multiple adipokines undoubtedly provides a powerful tool to study obesity. The area of obesity research is getting hotter ever over the past years. One of the key driving factor is that adipose tissue is found no longer to be an inert energy storage organ, but is emerging as an active participant in regulating physiological and pathologic processes. Many soluble factors have been identified from the adipose tissue and are so called as adipocytokines or adipokines. But adipokines are also expressed in a number of other tissues and organs. Because all of these factors can act in an autocrine, paracrine and endocrine manner in the organisms, adipokines are thought to serve as mediators linking obesity, inflammation, immunity and other obesity related diseases¹²⁻¹⁵.

Without doubt, simultaneous detection of multiple adipokines provides a powerful tool to study adipokines.

Here's how it works



II. Materials Provided

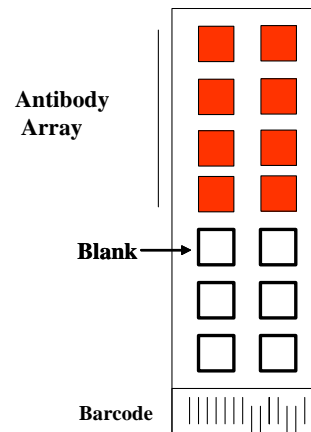
Upon receipt, all components of the RayBio[®] Human Adipokine Antibody Array kit should be stored at -20⁰C. At -20⁰C the kit will retain complete activity for up to 6 months. Once thawed, the glass chips, Fluorescent dye-streptavidin, internal control and 2X Blocking Buffer should be kept at – 20⁰C and all other component should be stored at 4⁰C. Use within three months after reagents have been thawed. Please use within six months of purchase.

- RayBio[®] Human Adipokine Antibody Microarray slides (4 subarrays in each glass chip)
- Biotin-Conjugated Anti-Adipokines (1 tubes, each tube for 4 wells)
- 1,500X Fluorescent dye-Conjugated Streptavidin (cy3 equivalent, 1 tube)
- 2X Blocking Buffer (5 ml)
- 20X Wash Buffer I (30 ml)
- 20X Wash Buffer II (30 ml)
- Internal control (powder, 1 tube)
- 2X Cell Lysis Buffer (10 ml)
- RayBio[®] G series antibody array accessory (including slide incubation chamber, Gasket, Protective cover, Snap-on sides and adhesive film)
- Manual

Additional Materials Required

- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- Plastic box

Layout of G series



8 arrays in one glass chip

III. Overview and General Considerations

A. Preparation of Samples

- Use serum-free conditioned media if possible.
- If serum-containing conditioned media is required, use serum as control since many types of sera contain adipokines.
- For cell lysates and tissue lysates, we recommend using 1X Cell Lysis Buffer to extract proteins from cell or tissue (e.g. using homogenizer). After extraction, spin the sample and save supernatant for experiment. Determine protein concentration. Dilute 2X Cell Lysis Buffer with H₂O (we recommend adding proteinase inhibitors to Cell Lysis Buffer before use). Prepare relative concentrated lysate since we recommend diluting lysate at least 10 folds with Blocking Buffer for array assay.
- We recommend using
 - 50 to 100 µl of cell culture supernatant
 - or 50 to 100 µl of original or 5-fold diluted serum or plasma
 - or 20-200 µg of protein for cell lysates and tissue lysates.

If you experience high background, you may further dilute your sample.

B. Handling glass chips

- The microarray slides are sensitive, do not touch the surface. Grip the slides by the edges only.
- Handle all buffers and slides with latex free gloves.
- Avoid breaking glass slide.
- Handle glass chip in clean environment.

C. Incubation

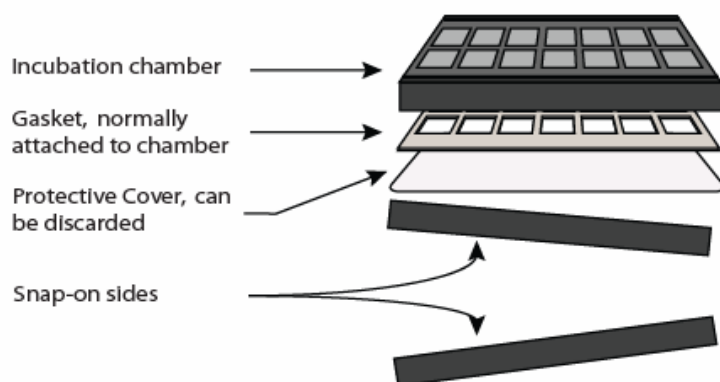
- Completely cover array area with sample or buffer during incubation, and cover the incubation chamber with adhesive film or plastic sheet protector to avoid drying.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or 50 μ l of sample or reagent is used.
- Avoid cross-contamination from overflowing solution to neighboring wells.
- Several incubation steps such as step 3 (blocking), step 4 (sample incubation), step 9 (biotin-Ab incubation) or step 12 (Fluorescent dye-streptavidin incubation) may be done at 4⁰C for overnight. Please make sure to cover the incubation chamber tightly to prevent evaporation.

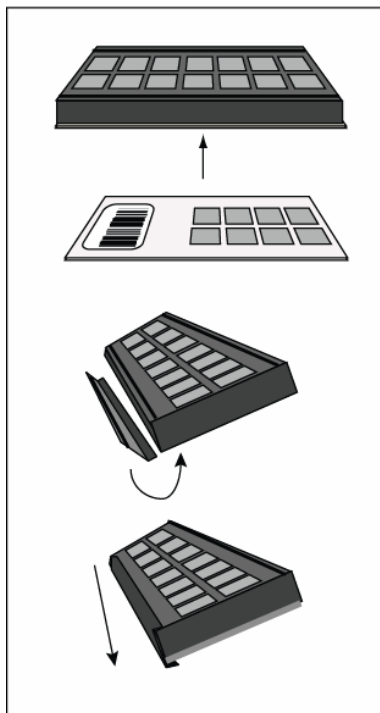
IV. Protocol

A. Blocking and Incubation

1. Take the glass chip out from the box. Let air dry for 2 hours.
2. Assemble the glass chip into incubation chamber and incubation frame as shown below. (Note: if you slide has be assembled, you can go to step 3 directly).

Instructions for incubation chamber assembly ***G Series and Q series arrays***





1 Carefully place slide at bottom of the chamber as shown. The slide will adhere somewhat to the bottom. Warning: the slide is fragile, so do not apply more than gentle force to the apparatus.

2 While gently holding chamber and slide, place side on chamber as shown, beginning with bottom flap first.

3 Then, press the top of the side into groove on chamber, and then apply even, gentle pressure from one end to the other. Repeat this procedure with the other side.

3. Add 100 μl 1 X Blocking Buffer into each well and incubate at room temperature for 30 min to block slides. Dilute 2X Blocking Buffer with H_2O . Make sure no bubbles are in the well.

Note: only add reagents to wells printed with antibodies.

4. Decant Blocking Buffer from each well, and incubate arrays with sample at room temperature for 1 to 2 hours. Dilute sample using 1X Blocking Buffer if necessary. We strongly recommend including Internal Control in your assay, add 100 μl of Blocking Buffer to IC tube, mix well and transfer 1 μl of IC to each well (50 to 100 μl of sample).

*Note: We recommend using 50 to 100 μl of conditioned media or 50 to 100 μl of original or 2-5 fold diluted serum or plasma or 10-200 μg of protein for cell lysates and tissue lysates. **Dilute the lysate at least 10 folds with 1 X blocking buffer to make a total volume of 50 to 100 μl . Make sure there is no bubble in the wells.***

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

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

Note: when transfer IC, use 0.1 μ l to 2 μ l pipettor.

5. Decant the samples from each well, and wash 3 times with 150 μ l of 1X Wash Buffer I at room temperature with gentle shaking. 2 min per wash. Dilute 20X Wash Buffer I with H₂O. Completely remove wash buffer I in each wash step.

Note: avoid solution flowing into neighboring wells.

6. 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 at room temperature with gentle shaking for 20 min.
7. Decant the 1X Wash Buffer I from each well, Put the glass chip with frame into the box with 1X 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. Remove all of Wash Buffer II in the well. Dilute 20X Wash Buffer II with H₂O.

8. Prepare working solution for biotin-conjugated antibodies. After brief spinning,

Add 300 μ l of 1x blocking buffer to the Biotin-Conjugated Antibody tube. Mix gently.

Note: the diluted biotin-conjugated antibodies can be stored at 4^oC for 2-3 days.

9. Add 70 μ l of diluted biotin-conjugated antibodies to each corresponding well. Incubate at room temperature for 2 hours.

Note: incubation may be done at 4^oC for overnight.

10. Wash as directed in steps 5 and then wash 3 times with 150 μ l of 1X Wash Buffer II at room temperature with shaking. 2 min per wash. Completely remove wash buffer II in each wash step.
11. Add 70 μ l of 1,500 fold diluted Fluorescent dye-conjugated streptavidin (after brief spinning, add 1.5 ml of Blocking Buffer to

Fluorescent dye-conjugated streptavidin tube) to each subarray. Cover the incubation chamber with Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in dark room.

12. Incubate at room temperature for 1 to 2 hours.

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

13. Wash with Wash Buffer I **twice** as directed in steps 5.

B. Fluorescence Detection

1. Decant excess Wash Buffer from wells.
2. Disassemble the slide out of the incubation frame and chamber.
3. Place the whole slide in 50 ml centrifuge tube, add enough Wash Buffer I (about 30 ml) to cover the whole slide and gently shake at room temperature for 10 minutes. Decant Wash Buffer I. Repeat Wash Buffer I once. Wash with Wash Buffer II (about 30 ml) with gentle shake at room temperature for 10 minutes. Or wash using slide chamber. Rinse the slide with distilled H₂O.
4. Remove water droplets by centrifuge at 1,000 rpm for 3 minutes and then let slide dry completely in air at least 20 minutes (protect from light). Make sure the slides are absolutely dry before the scanning procedure.
5. Image the signals using laser scanner such Axon GenePix using cy3 channel.

Note: we recommend scanning slides right after experiment. You also can store the slide at -20⁰C in dark for several days. If you do not have a laser scanner, we can provide service for you. Just simply send your slide to us and we will take care of it.

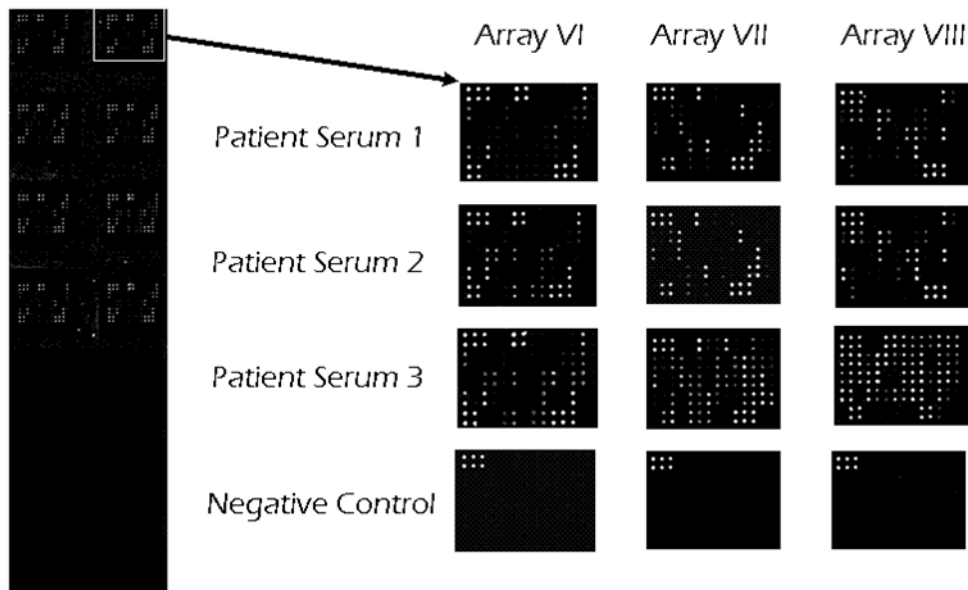
V. Interpretation of Results:

The following figure shows RayBio[®] Human Cytokine Antibody Array G series 2000 probed with different cell culture supernatant. The images were captured using laser scanner. The biotin-conjugated protein produces positive signals, which can be used to identify the orientation and to compare the relative expression levels among the different wells. The internal control (IC) can also be used to normalize the signal intensities among array membranes in different experiments.

The signal intensities obtained from laser scanner can simply be imported into our analysis tool. The analysis tool will help you:

- Locate your signal intensities to antibody array map
- Link the protein to website for more detailed information on the particular protein
- Protein list sorting
- Average signal intensities
- Subtract background
- Normalize the data from different samples
- Obtain protein level comparison charts among different samples

This analysis tool is very simple and affordable, which will not only assist in compiling and organizing your data, but also reduces your calculations to a “copy and paste” step.



If you do not use our **RayBio® Analysis Tool**, you can locate the cytokines by referring to corresponding RayBio® Human cytokine Antibody Array G series map.

Normalization and comparison

For biomarker discovery or for analysis of large number of arrays, great attention must be paid to the normalization. Our antibody array design includes several controls for normalization and comparison of arrays performing in different membranes and different experiments (for more information please read the reference 17).

Positive control. Positive control is biotinylated protein. It can be used to normalize the streptavidin incubation step. If the positive signals from different array membranes are similar, positive control is a simple and effective way for normalization.

Internal control. RayBio® antibody arrays also include spiking-in protein serving as internal control (IC). The spiking-in proteins do not have cross-reactivity with protein in the array. It can be used to normalize the entire process.

Negative control. Negative control is BSA. Normally, it should only give a background reading.

RayBio® Human Adipokine Antibody Arrays 1
(for simultaneous detection of 62 human adipokines)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
a	POS 1	POS 2	POS 3	NEG	4-1BB	ACE-2	Acrrp30	Adipsin	AgRP	Angiopoietin-1	Angiopoietin-2	ANGPTL4	CRP	ENA-78
b	POS 1	POS 2	POS 3	NEG	4-1BB	ACE-2	Acrrp30	Adipsin	AgRP	Angiopoietin-1	Angiopoietin-2	ANGPTL4	CRP	ENA-78
c	Fas	FGF-6	Growth Homon	HCC-4	IFN-gamma	IGFBP-1	IGFBP-2	IGFBP-3	IGF-I	IGF-1 sR	IL-1 alpha	IL-1 beta	IL-1 sRI	IL-1 R4/ST2
d	Fas	FGF-6	Growth Homon	HCC-4	IFN-gamma	IGFBP-1	IGFBP-2	IGFBP-3	IGF-I	IGF-1 sR	IL-1 alpha	IL-1 beta	IL-1 sRI	IL-1 R4/ST2
e	IL-6	IL-6 sR	IL-8	IL-10	IL-11	IL-12	Insulin	IP-10	Leptin	Leptin R	LIF	Lymphotoctin	MCP-1	MCP-3
f	IL-6	IL-6 sR	IL-8	IL-10	IL-11	IL-12	Insulin	IP-10	Leptin	Leptin R	LIF	Lymphotoctin	MCP-1	MCP-3
g	MCSF	MIF	MIP-1beta	MSP alpha	OPG	OSM	PAI-I	PARC	PDGF-AA	PDGF-AB	PDGF-BB	RANTES	Resistin	Serum Amyloid A
h	MCSF	MIF	MIP-1beta	MSP alpha	OPG	OSM	PAI-I	PARC	PDGF-AA	PDGF-AB	PDGF-BB	RANTES	Resistin	Serum Amyloid A
i	SDF-1	sTNT RI	sTNF RII	TECK	TGF-beta	TIMP-1	TIMP-2	TNF-alpha	VEGF	XEDAR	IC 1	IC 2	IC 3	NEG
j	SDF-1	sTNT RI	sTNF RII	TECK	TGF-beta	TIMP-1	TIMP-2	TNF-alpha	VEGF	XEDAR	IC 1	IC-2	IC 3	NEG

We also offer Custom Human Cytokine Antibody Arrays. You can select the cytokines of interest from the following list and we will produce the customized array at an affordable price. For more information, please visit our website, www.raybiotech.com.

Note: IL-12 reacts both IL-12p40 and IL-12p70. IL-12p70 only recognizes IL-12p70.

Abbreviations: IP-10, Interferon-inducible protein-10; LAP, latency associated peptide (TGF- β 1); LIF, leukocyte inhibitory factor. MMP, Matrix Metalloproteinase; Pos, positive control; Neg, negative control. All other are used standard abbreviations.

4-1BB/ TNFRSF9	6Ckine	ACE-2	Activin A	adiponectin/ Acrp30	Adipsin	AgRP(ART)
ALCAM	ANGIOGENIN	Angiopoietin-1	Angiopoietin-2	Angiostatin	ANGPTL4	AR (amphiregulin)
Axl	B2M	B7-1(CD80)	BCAM	BDNF	bFGF	BLC
BMP-4	BMP-5	BMP-6	BMP-7	b-NGF	BTC	Cardiotrophin-1
Cathepsin S	CCL28/VIC	CD14	CD27	CD30	CD40	CD40 Ligand
Ck beta 8-1	CNTF	CRP	CTACK/CCL27	CTLA-4	CXCL16	DAN
Dkk-1	Dkk-4	DR6	Dtk	E-Cadherin	EGF	EGF R
ENA-78	Endoglin(CD105)	Endostatin	Eotaxin	Eotaxin-2	Eotaxin-3	EPO R
ErbB3	E-Selectin	Fas Ligand	Fas/TNFRSF6	Fcr RII	FGF-4	FGF-6
FGF-7	FGF-9	Flt-3 Ligand	Follistatin	Fractalkine	GCP-2	GCSF
GDNF	GITR Ligand/ TNFSF18	GITR/ TNFRF18	GM-CSF	GRO	GRO alpha	Growth Hormone
HB-EGF	HCC-1	HCC-4/CCL16	HGF	HVEM	I-309	ICAM-1
ICAM-2	ICAM-3	IFN-gamma	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4
IGFBP-6	IGF-I	IGF-I SR	IGF-II	IL-1 alpha	IL-1 beta	IL-1 R4/ST2
IL-1 sRI	IL-1 sRII	IL-10	IL-10 R alpha	IL-10 R beta	IL-11	IL-12
IL-12 p40	IL-12 p70	IL-13	IL-13 R alpha	IL-15	IL-16	IL-17
IL-17B	IL-18	IL-18 BP alpha	IL-18 R alpha	IL-18 R beta	IL-1ra	IL-2
IL-2 R alpha	IL-2 R beta	IL-2 R gamma	IL-2 sR alpha	IL-21 R	IL-28A	IL-3
IL-4	IL-5	IL-5 R alpha	IL-6	IL-6 sR	IL-7	IL-8
IL-9	IL-9 R	Insulin	IP-10	I-TAC/ CXCL11	LAP (TGF-beta 1)	Leptin R
LEPTIN (OB)	LIF	LIGHT	L-Selectin	Lymphotoxin	MCP-1	MCP-2
MCP-3	MCP-4	M-CSF	M-CSF R	MDC	MICA	MICB
MIF	MIG	MIP-1 alpha	MIP-1 beta	MIP-1delta	MIP-3 alpha	MIP-3 beta
MMP-1	MMP-10	MMP-13	MMP-2	MMP-3	MMP-8	MMP-9
MPIF-1	MSP alpha	NAP-2	NGF R	NT-3	NT-4	ONCOSTATIN M
Osteoprotegerin	PAI-1	PARC	PDGF R alpha	PDGF R beta	PDGF-AA	PDGF-BB
PECAM-1	PF4	PIGF	Prolactin	P-selectin	RAGE	RANK
RANKL (TRANCE)	RANTES	Resistin	SAA	SCF	SCF R	SDF-1
SDF-1 beta	sgp130	Siglec-5	ST2	sTNT RI/TNFRS1A	sTNF RII/TNFRS1B	Tarc
TECK/ CCL25	TGF-alpha	TGF-beta	TGF-beta 2	TGF-beta 3	Tie-1	Tie-2
TIMP-1	TIMP-2	TIMP-3	TIMP-4	TNF-alpha	TNF-beta	TPO
TRAIL R1	TRAIL R2	TRAIL s R3/TNFRS10C	TRAIL s R4/TNFRS10D	TREM-1	u PAR	VCAM-1
VE-Cadherin	VEGF	VEGF R2	VEGF R3	VEGF-D	VEGI	XEDAR

RayBiotech, Inc., the protein array pioneer company, strives to research and develop new products to meet demands of the biomedical community. RayBio's patent-pending technology allows detection of over 180 cytokines, chemokines and other proteins in a single experiment. Our format is simple, sensitive, reliable and cost effective. Products include: Cytokine Arrays, Chemokine Arrays, ELISA kits, Phosphotyrosine kits, Recombinant Proteins, Antibodies, and custom services.

1. Antibody arrays

- Cytokine antibody array

 - Human cytokine antibody arrays

 - Mouse cytokine antibody arrays

 - Rat cytokine antibody arrays

- Pathway- or disease-focused antibody arrays

 - Inflammation antibody array

 - Angiogenesis antibody array

 - Chemokine antibody array

 - Growth factor antibody array

 - MMP antibody array

 - Atherosclerosis antibody array

- Quantibody arrays for quantitative measurement of cytokine and other protein concentration

- Phosphorylation antibody arrays

- Biotin label-based antibody arrays for high density antibody arrays.

- Antibody analysis tool, software

2. ELISA
3. Cell-based phosphorylation assay
4. Custom antibody arrays
5. Antibody
6. Recombinant protein
7. Peptide
8. Protein arrays

RayBiotech also provides excellent custom service:

1. Antibody arrays
2. Protein arrays
3. Peptide synthesis
4. Production of recombinant protein and antibody
5. Peptide arrays

6. Phosphorylation arrays
7. ELISA

Just simply send your samples and we will do the assay for you.

Technology transfer program

Have you developed technologies or reagents interested to the scientific and research community? RayBiotech can help you commercialize your technologies, reagents and dream.

VI. 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	Excess of biotinylated antibodies	Make sure correct amount of antibodies
	Excess of streptavidin	Make sure correct amount of streptavidin
	Inadequate detection	Check laser power And PMT parameters
	dust	Work in clean environment
	Insufficient wash	Increase wash time and use more wash buffer
Uneven signal	Bubbles formed during incubation	Avoid bubble formation during incubation
	Arrays are not completed Covered by reagent	Completely cover arrays with solution

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Note:

RayBio[®] is the trademark of RayBiotech, Inc.

Cytokine protein arrays are RayBiotech patent-pending technology.

This product is intended for research only and is not to be used for clinical diagnosis. Our products may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech, Inc.

Under no circumstances shall RayBiotech be liable for any damages arising out of the use of the materials.

Products are guaranteed for three months from the date of purchase when handled and stored properly. In the event of any defect in quality or merchantability, RayBiotech's liability to buyer for any claim relating to products shall be limited to replacement or refund of the purchase price.

This product is for research use only.



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