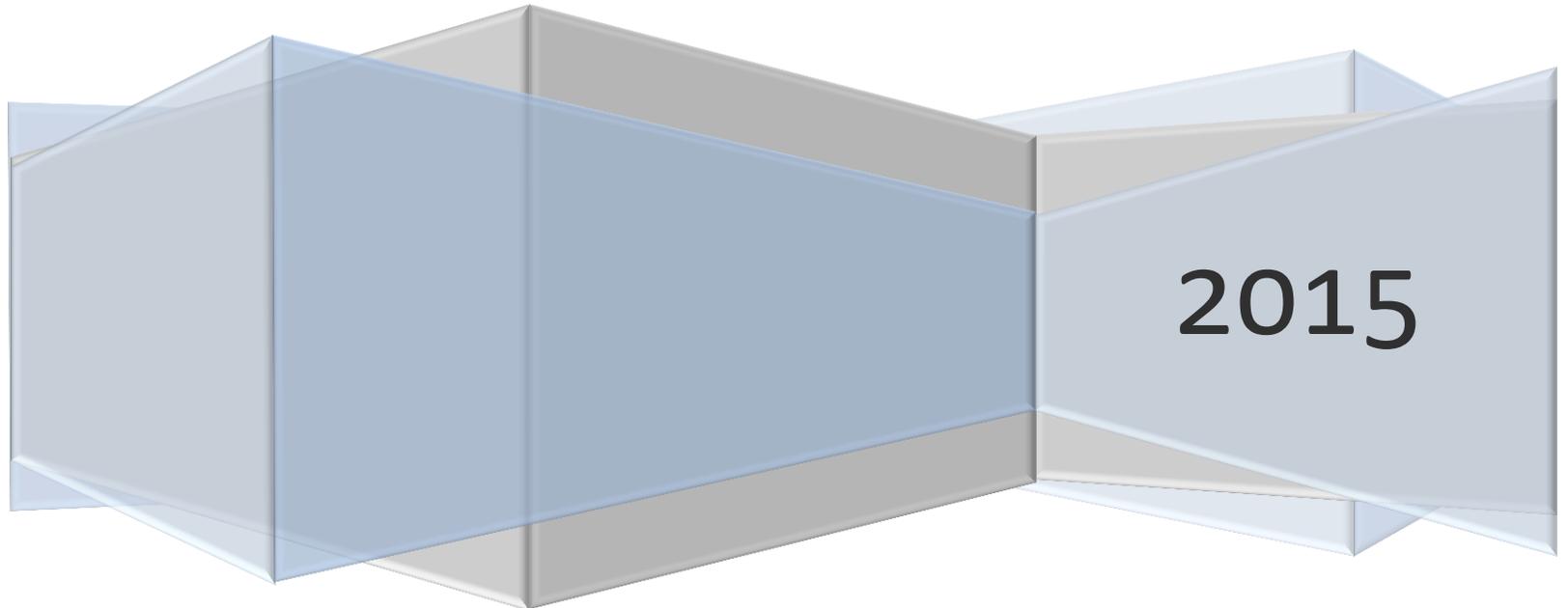


Protein/Cytokine Array Services

at the **Analytical Instrumentation Core**

Boston University, AIC, DOM, BMC, BUMC



2015

Analytical Instrumentation Core Mission:

The AIC supports investigators through both cutting-edge analytical instruments and services that facilitate research and education in the Department of Medicine, Boston University School of Medicine, and Boston Medical Center. Our goals are to:

1. Maintain core instruments
2. Train investigators how to use equipment and to help design experiments
3. Share the resources and increase accessibility of instruments for investigators
4. Introduce innovative analytical instrumentation by regularly holding seminars
5. Support investigators in their efforts to win extramural research funding

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Introduction to Protein/Cytokine Array

Services at the Analytical Core:

The Analytical Instrumentation Core now offers an Array Service for measuring proteins, cytokines and biomarkers through the use of multiplexing technology. Researchers are able to submit a wide variety of sample types that range from plasma/serum to cell supernatants and test up to a maximum of 50 analytes at a time (multiplexing) in each well on a 96-well microplate. Including standards and quality controls up to 40 samples can be run in duplicate on each plate.

Protein Arrays are useful for:

- **Verifying that changes at the mRNA level (gene expression) correspond to changes at the protein level**
- **Preliminary (fishing) studies to screen for a large number of proteins/biomarkers**
- **Screening multiple proteins simultaneously to reduce sample volume needed and costs**



Aushon
Shaping the future of ELISA



Advantages of Using the Array Service

The Protein Array Service is cost effective and great for researchers!

- Multiplexing allows for the detection of up to 50 analytes at a time (No need to have to run multiple ELISAs)
- Enables researchers to extract more data from a given amount of sample in the same amount of time as a single-plex ELISA assay
- Arrays offer a large dynamic range of detection (based on either fluorescence or chemiluminescence) with high precision and low background, eliminating the need to run several dilutions
- The service is performed by AIC professionals with consistency and accuracy
- AIC acts as liaison between array companies and BU researchers, allowing for the lowest prices on plates, supplies, and reagents.
- Researchers are able to get results in as little as one week, depending on the scope of the project and the number of plates being analyzed.



What exactly are Protein Arrays?

Protein arrays are an extension of PCR Arrays that are used to verify that changes at the mRNA level truly correspond to changes at the protein level. In addition, protein arrays can be used on a wide variety of samples for detecting biomarkers in diseased versus wild-type samples. They rely on the use of fluorescent magnetic beads, electrochemiluminescence or chemiluminescence to detect the presence of a particular protein or biomarker. Currently, we have the following 3 platforms available that can be used to detect proteins:



Aushon Cirascan (Chemiluminescence)



MSD Sector (Electrochemiluminescence)

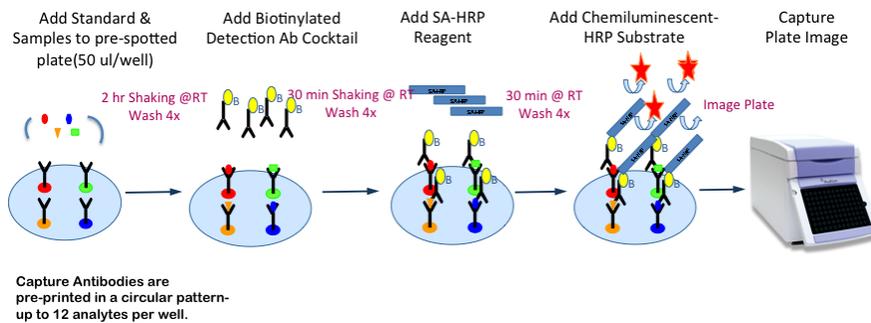


Luminex Magpix (Fluorescent Magnetic Beads)

How do these Protein Arrays Work?

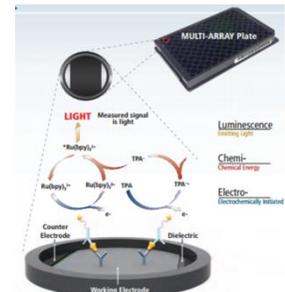
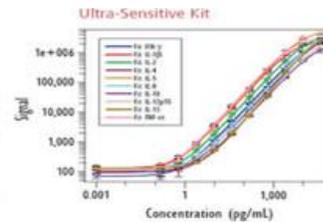
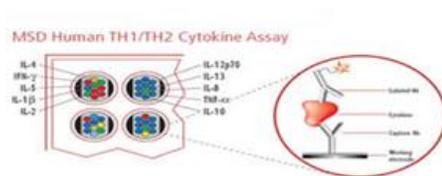
1. Aushon Workflow

CiraPlex arrays are precisely printed in a circular pattern, allowing for up to 12 analytes per well. This design requires less sample volume per test than ELISA and provides significantly improved sensitivity, reproducibility, dynamic range, and accuracy.



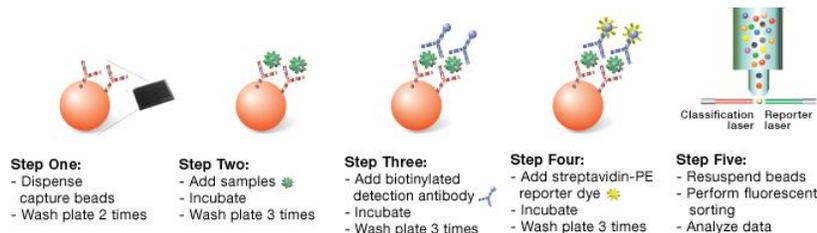
2. Meso Scale Discovery (MSD) Workflow

The surface of each well is coated with a carbon electrode for greater binding and to allow for electrical stimulation. Like ELISAs, it uses both capture and detection antibodies to detect the protein of interest. Detection antibodies emit light when electrochemically stimulated and is captured by the instrument.



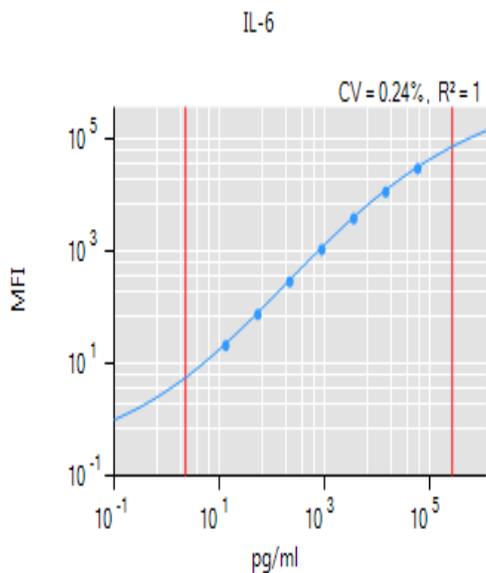
3. Luminex Workflow

Detection of proteins/cytokines occurs on micron-sized magnetic beads which contain capture antibodies. Each bead contains a unique blend of fluorophores that acts as a signature and is associated with a single analyte. Up to 50 different bead sets can be combined in a single well.

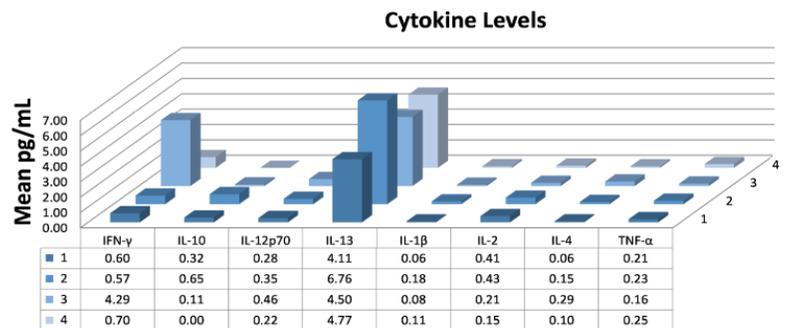


What do the Results look like?

Data-analysis software and the ability to save results as both Excel-based and PDF files allow for quick and easy interpretation of array data. The software included on each platform is able to use the information generated from the standard curves to quantitatively determine the amount of protein (concentration) that is in a given sample. Finally, the software is then able to organize all of the data into plots and charts, which can immediately be integrated into a publication, poster, or presentation.



Standard Curve for IL-6



Bar Graph of Mean Cytokine Levels

Sample Name	hIFNg	hIL10	hIL12p70	hIL1a	hIL1b	hIL2	hIL6	hIL8	hTNFa
	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
CONTROL (3)	131.3	142.4	203.0	100.7	60.4	120.3	59.7	85.5	806.9
CONTROL (12)	111.6	120.4	181.1	116.8	60.2	128.0	57.0	108.6	720.7
Sample 01	428.7	412.1	743.3	452.1	209.9	431.4	220.1	428.6	2656.3
Sample 02	416.1	409.5	601.8	361.7	188.5	427.1	182.0	374.1	2724.4
Sample 03	427.7	442.5	649.1	423.8	212.8	418.8	202.4	387.0	2769.6
Sample 04	412.7	369.6	626.4	398.8	193.6	361.0	179.4	380.0	2457.4
Sample 05	384.3	327.3	576.2	389.1	195.8	405.0	197.9	354.1	2074.0
Sample 06	402.7	383.7	569.1	324.6	218.1	468.8	215.4	372.0	2480.9
Sample 07	458.8	572.2	852.4	521.2	358.8	743.7	416.8	740.0	3871.3
Sample 08	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Summary Table of Analyte Concentrations

Catalogued Arrays Available

There are over 110 cataloged arrays available from various manufacturers including EMD Millipore, Bio-Rad, Life Technologies, R&D Systems and many more! Arrays are available for a wide variety of species including human, rat, mouse, porcine, primate and bovine!

Please feel free to let us know which particular analytes you are interested in and what type of sample you have and we would be more than happy to find the perfect kit for you!

Example of Some Catalogued Kits Available:

Bone Metabolism

Cancer Biomarkers

Cardiovascular

Immunology/Immune Response

Metabolism/Endocrinology

Neuroscience

Cell Signaling

Toxicity



Custom Arrays

If you have a specific list of proteins that you would like to analyze that are not available on one of the Catalogued Array Kits there is also an option for a **Custom Array**. Custom Arrays allow for the analysis of up to 50 different analytes of your choice so long as the analytes are compatible and show no cross-reactivity. However, Custom Arrays are more expensive than the catalogued arrays because of design, test and setup fees. In addition, the kits generally take longer to arrive and usually require a researcher to purchase a minimum number. If you are interested in a Custom Array please do not hesitate to contact us for exact pricing and further details!

Cira™ Custom Kits



Custom Offering***

Within a species, order any combination of analytes for a custom plex of up to 12 analytes. Contact Aushon Customer Support to determine if your desired plex is feasible based on required dilution factors and known cross reactivity data. Take a look at your options below: **A total of 244 Analytes!**

Human Analytes	IL-1β	NGAL	Mouse Analytes	GRO/KC
α2-Macroglobulin	IL-1ra	NT3	CNTF	IFNγ
Acrop-30 (Adiponectin)	IL-2	NT-proBNP	CRP (C-reactive protein)	IL-1α
Ang-1	IL-2Rα	OPN (Osteopontin)	Eotaxin	IL-1β
Ang-2	IL-2Rγ	OPN (Osteopontin)	GM-CSF	IL-2
Apo A-1	IL-3	PAI-1 Active	ICAM-1	IL-4
Apo B-100	IL-4	PAI-1 Total	IFNγ	IL-6
AR (Amphiregulin)	IL-5	PAPPA	IL-1α	IL-10
BAFF	IL-6	PD-1	IL-1β	MCP-1
BCA-1 (CXCL13)	IL-6R	PDGF-AA	IL-1ra	MIP-1α
β-NGF	IL-7	PDGF-AB	IL-2	MIP-2
BDNF	IL-8	PDGF-BB	IL-4	MIP-3α
BD-2	IL-10	PEDF	IL-5	RANTES
BMP-9	IL-12p40 (p40/homodimer)	PLGF (Placental Growth Factor)	IL-6	TGFB1*
Cathepsin-D	IL-12p70 (heterodimer)	Proctectin	IL-10	TNFα
CD14	IL-13	P-Selectin	IL-12p40	
CD40L	IL-15	RAGE	IL-12p70	
Clusterin	IL-17A	RANKL	IL-13	Porcine Analytes
CNTF	IL-17E	RANTES	IL-17	IL-13
COX-2	IL-18**	RBP4*	IL-18**	IFNγ
C-peptide	IL-23	Resistin	IP-10	IL-1β
hsCRP (C-reactive protein)	Insulin	SAA	KC	IL-4
E-Cadherin	IP-10	SCF	KDR/VEGF-R2	IL-6
EGF	ITAC	SDF-1	L-Selectin	IL-8
EGFR	KGF	TARC	MCP-1 (JE)	IL-10
ENA-78	Leptin	TFF-3 (urine only)	MCP-5	IL-13
Endoglin	LIF	TGFα	MDC	TNFα
Eotaxin	L-Selectin	TGFβ1*	MIP-1α	
ER (Epregrulin)	Lymphotactin	TGFβ2*	MIP-1β	Primate Analytes
ErbB2	MCP-1	Thrombospondin	MIP-2	MIP-1α
E-Selectin	MCP-2	Tie-2 (Plasma only)	MIP-3β	IFNγ
FasL	MCP-3	TIM-1 (Urine only)	MMP-3	IL-1β
FGF basic	MCP-4	TIMP-1	MMP-9	IL-2
FGF receptor	IL-CSF	TIMP-2	OPN (Osteopontin)	IL-6
Fibrinogen	MDC	TNFα (Active Trimer)	PDGF-BB	IL-8
Fibronectin	G-CSF	TNFα (Monomer + Trimer)	P-Selectin	MCP-1
G-CSF	MIF	TNF-R1	RANTES	MIP-1α
GDNF	MIG	TNF-R2	SDF-1β	MIP-1β
GM-CSF	MIP-1α	TNF-R3	TARC	MMP-9
GRDα	MIP-1β	TSLP	TGFβ1*	RANTES
GRY	MIP-3α	TSP-1 (Thrombospondin-1)	TNFα	TNFα
HB-EGF	MIP-3β	TSP-2 (Thrombospondin-2)	TNF-R1	
HGF	MIP-4 (PARC)	TWEAK	VCAM-1	Bovine Analytes
HGH	MMP-1	VEGF	VEGF	IFNγ
I-309	MMP-2*	VEGF-C	VEGF-D	IL-1β
ICAM-1	MMP-3	VEGF-F1	VEGF-F2	IL-2
IFNα **	MMP-7	VEGF-F3	VEGF-F4	IL-4
IFNγ	MMP-8	VEGF-F5	VEGF-F6	IL-6
IGFBP-1	MMP-9	VEGF-F7	VEGF-F8	TNFα
IGFBP-2	MMP-10	VEGF-F9	VEGF-F10	
IGFBP-3	MMP-13	VEGF-F11	VEGF-F12	
IgE	MPO (Myeloperoxidase)	VEGF-F13	VEGF-F14	
IL-1α	NAP-2			

Custom V-PLEX Panel: Sub-plex of Pre-Configured Kit

Mouse V-Plex

- IFN-γ
- IL-1β
- IL-2
- IL-4
- IL-5
- IL-6
- IL-10
- IL-12p70
- KC/GRO
- TNF-α

1-3 day
shipment

1. Select Assays (on website)
2. Select V-PLEX Plus
3. Quote is generated.
4. Customer places order.
5. Fulfillment in 1-3 days
6. Customer gets:
 - ✓ Validated panel
 - ✓ Individual detection antibodies for flexibility
 - ✓ Fast delivery
 - ✓ Controls available
 - ✓ Certificate of Analysis

Proinflammatory Panel 1 (mouse)

*May not be combined in an array. ** Service Only ***Custom plexes are subject to design, test and setup fees as these are non-stocked items. N analytes will plex together due to endogenous protein levels and potential cross reactivity. Menu subject to change. Contact an Aushon Customer Specialist for more information about your desired plex (email sales@aushon.com, call 1-877-297-4664 or 978-436-6400).

Cost of Array Service

The **Basic Service** applies to researchers who wish to purchase the kits on their own (or through the Core) and perform their own sample preparation. The researcher is only looking to have their plate read on one of our available instruments.

Basic Service: Plate Reading Only

Instrument Usage = \$85 per plate

Total Cost = \$85 per plate

The **Full Service** applies to researchers who want the AIC to perform the sample preparation and plating for them. They will provide samples in the form of plasma, serum, supernatants, etc. to the core for further processing. The charges for the sample preparation are separate from the charges for reading the plate and the price of the kit. The overall cost will vary and depends on the number of plates run along with the number of analytes being analyzed.

Full Service: Sample Prep, Plate Reading and Data Analysis

Sample Preparation = ~\$265 per plate

Instrument Usage = \$85 per plate

Data Analysis (Optional) = \$70 per plate

Cost of the kit = Depends on the particular kit(s) purchased

Total Cost = \$420 per plate (Not including the cost of the kit(s))