

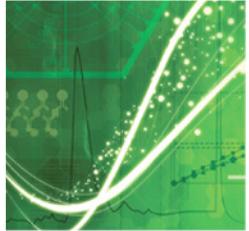
The Break Through of Automatic High Throughput Multi-targets Detection Technology

Finex Chiu 邱辰哲

產品專員

Bio-Rad Laboratories Ltd.



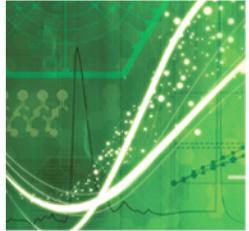


Break through topics

✓ **Multiplex**

✓ **High throughput**

✓ **Automatics**

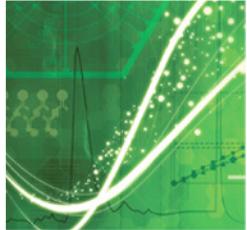


Break through topics

✓ **Multiplex**

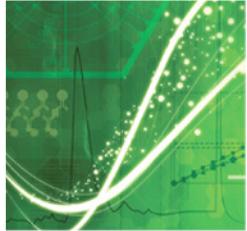
✓ **High throughput**

✓ **Automatics**

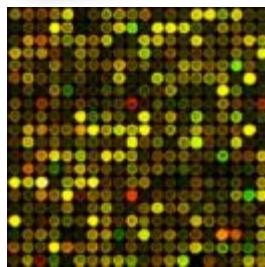


Why Multiplex?

- Decrease precious sample volume requirements
 - 12 ul sample for 100 data points
- Reduce assay reagent volume, expense and labor
- Include assay controls in each well, rather than on each plate
- Dramatically increase the amount of information from a single sample
- Better correlation of data between different targets (target analysis is simultaneous for each sample)
- Control the same interaction condition of the reaction



Key multiplex technology



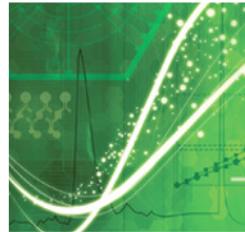
Microarray



Real Time PCR

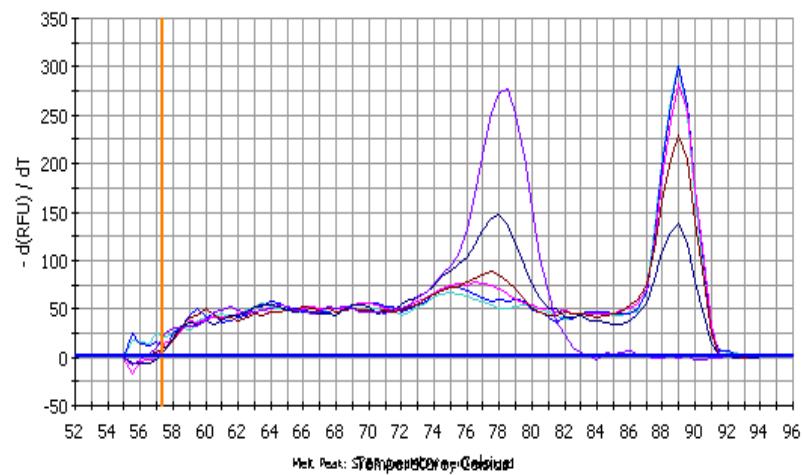
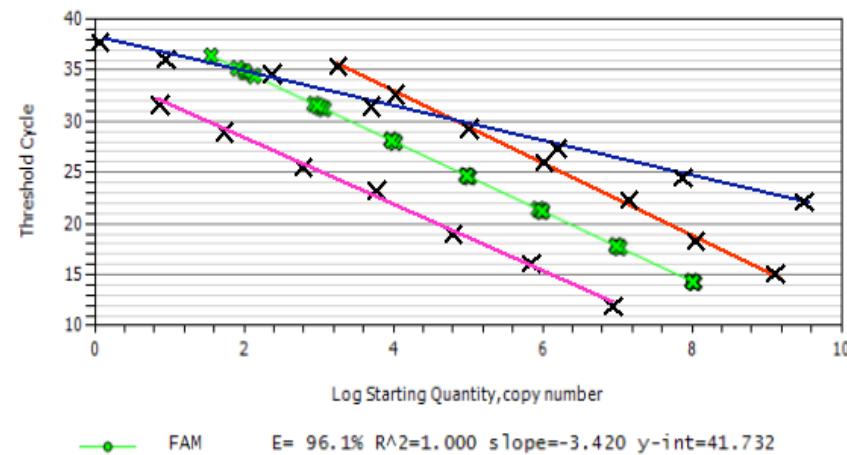
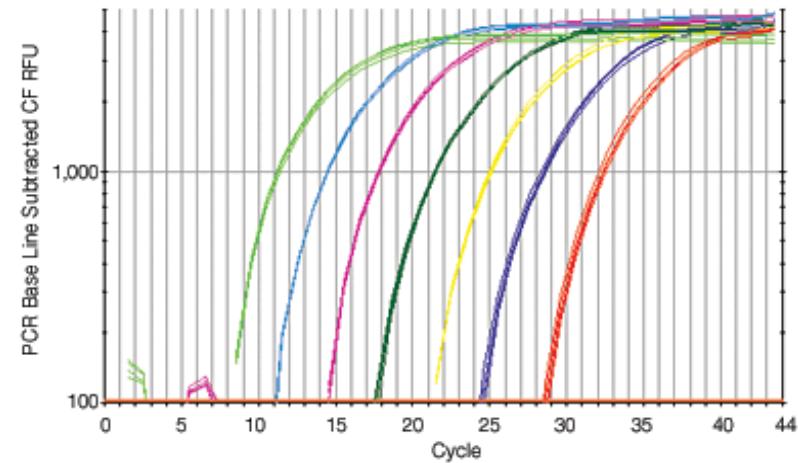


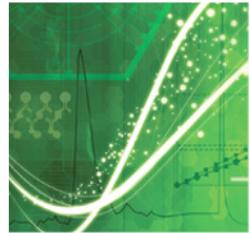
BioPlex Suspension Array



The bottle neck of QPCR

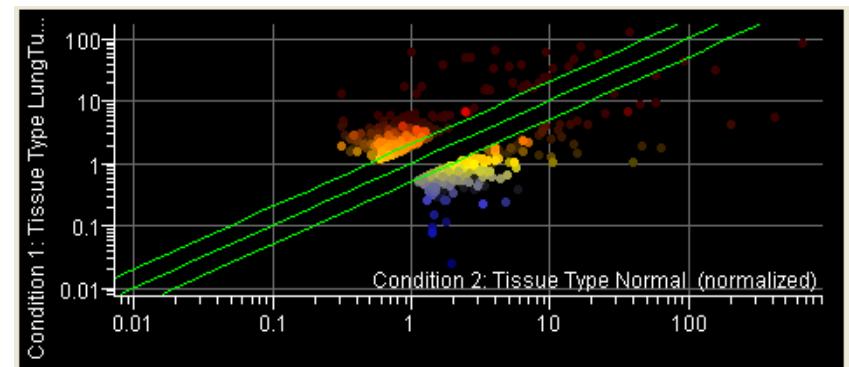
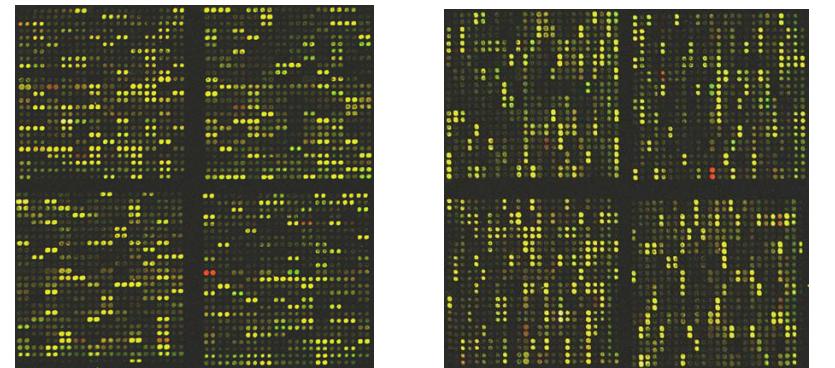
- Real Time PCR: Hot tool, easy to use
 - Up to five targets only
 - Sample preparation, NA extraction
 - Data Optimization
 - NAT only
 - False positive



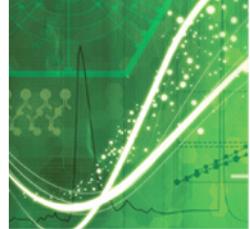


The bottle neck of micro array

- Micro array: High entrance barrier
 - One chip, one sample
 - Overnight incubation
 - Reproducibility
 - Fuzzy data
 - Database issue
 - QL only



Low density array / Strip



Super comparison matrix

Micro Array

VS

Suspension Array

- High density, >10K points
- Probe coated on solid phase
- Low reproducibility
- One array for one sample
- High entrance barrier
- Need other database
- High Cost
 - 1.5 working days
 - High cost of consumables

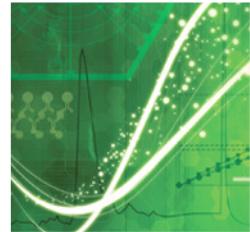
- Low density, up to 100 points
- Probe coated on mobile phase
- High reproducibility
- High throughput, up to 96 samples
- Easy to take over
- Easy to identify
- Lower Cost
 - 4 hours
 - Lower cost of consumables

What's Suspension Array?

Suspension Array = Beads Array = BioPlex \neq Micro Array

xMAP Technology

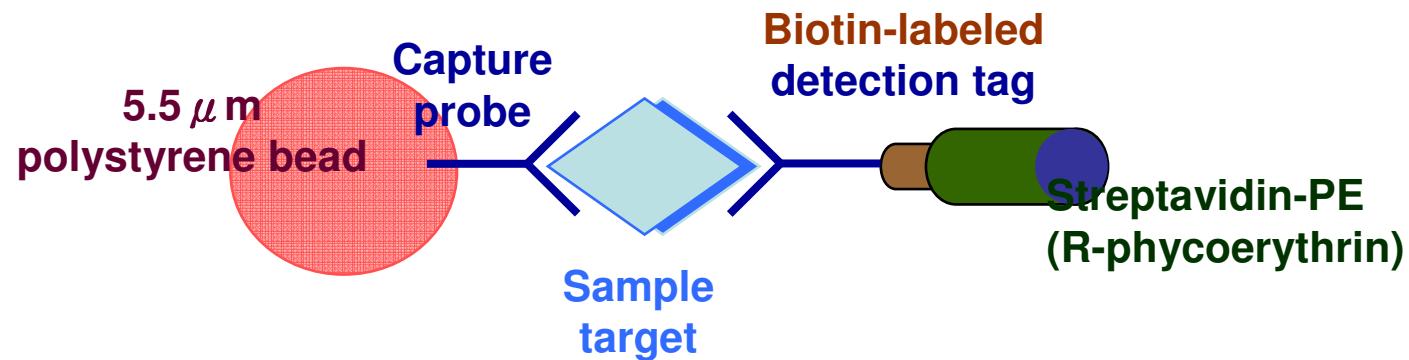


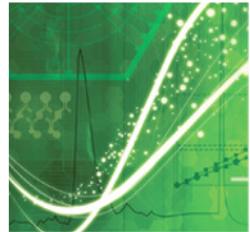


Suspension Array Technology

xMAP

flexible Multi-Analyte Profiling



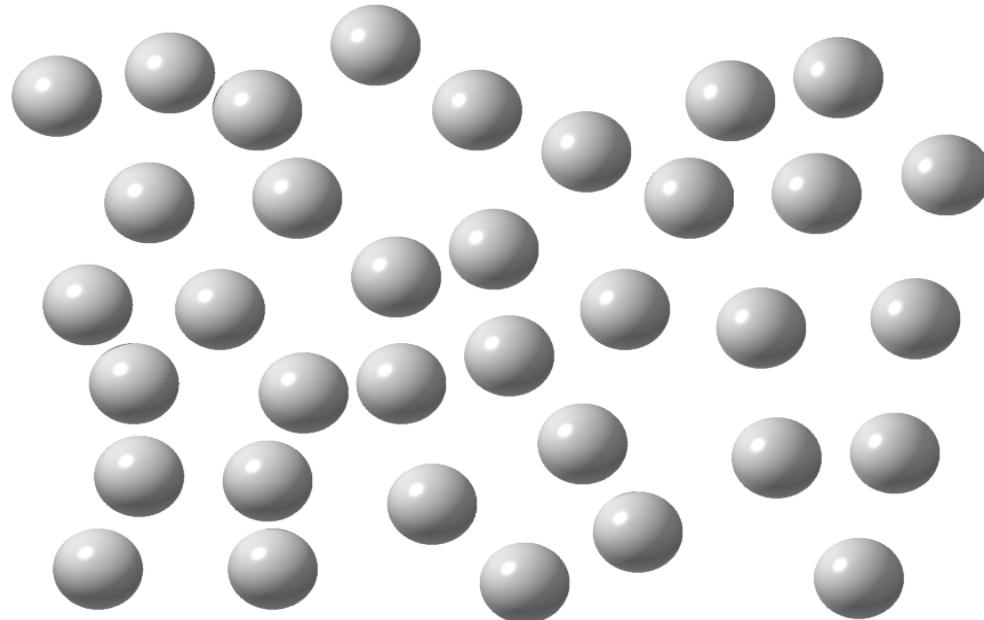


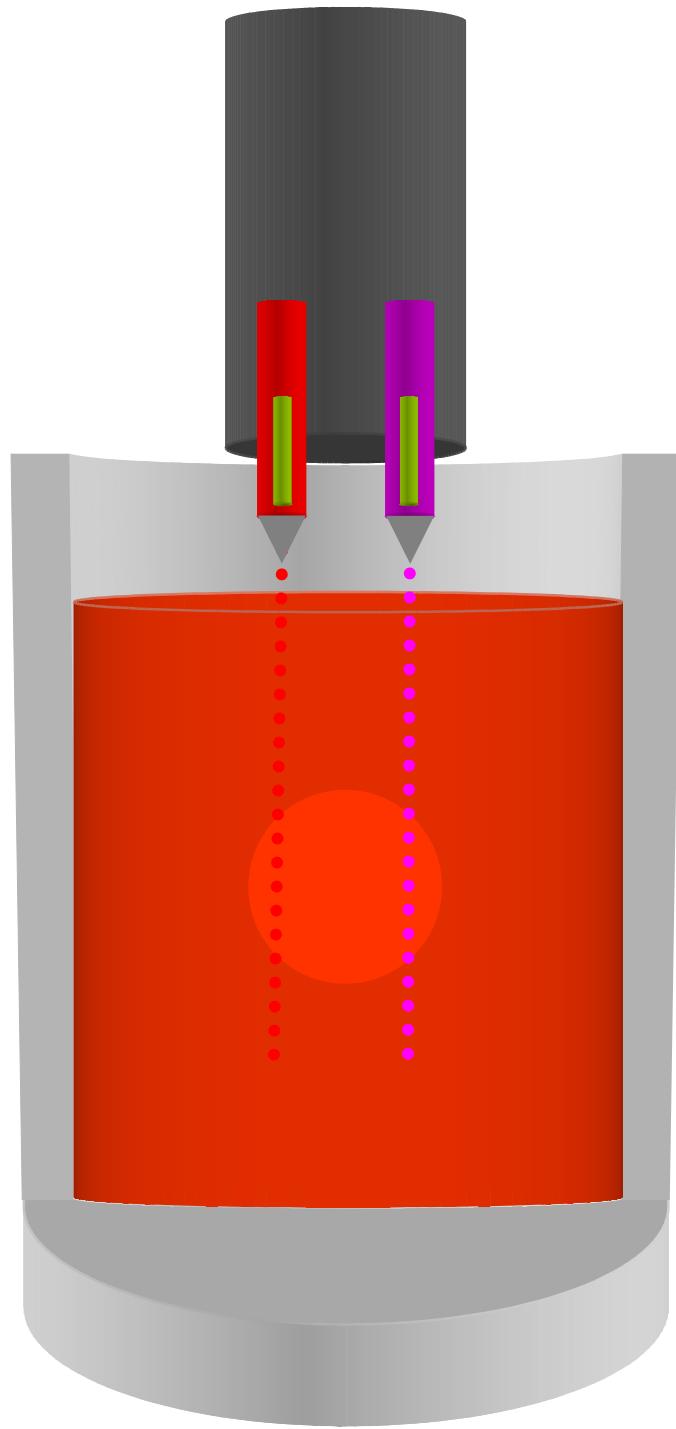
Suspension Bead Array Technology

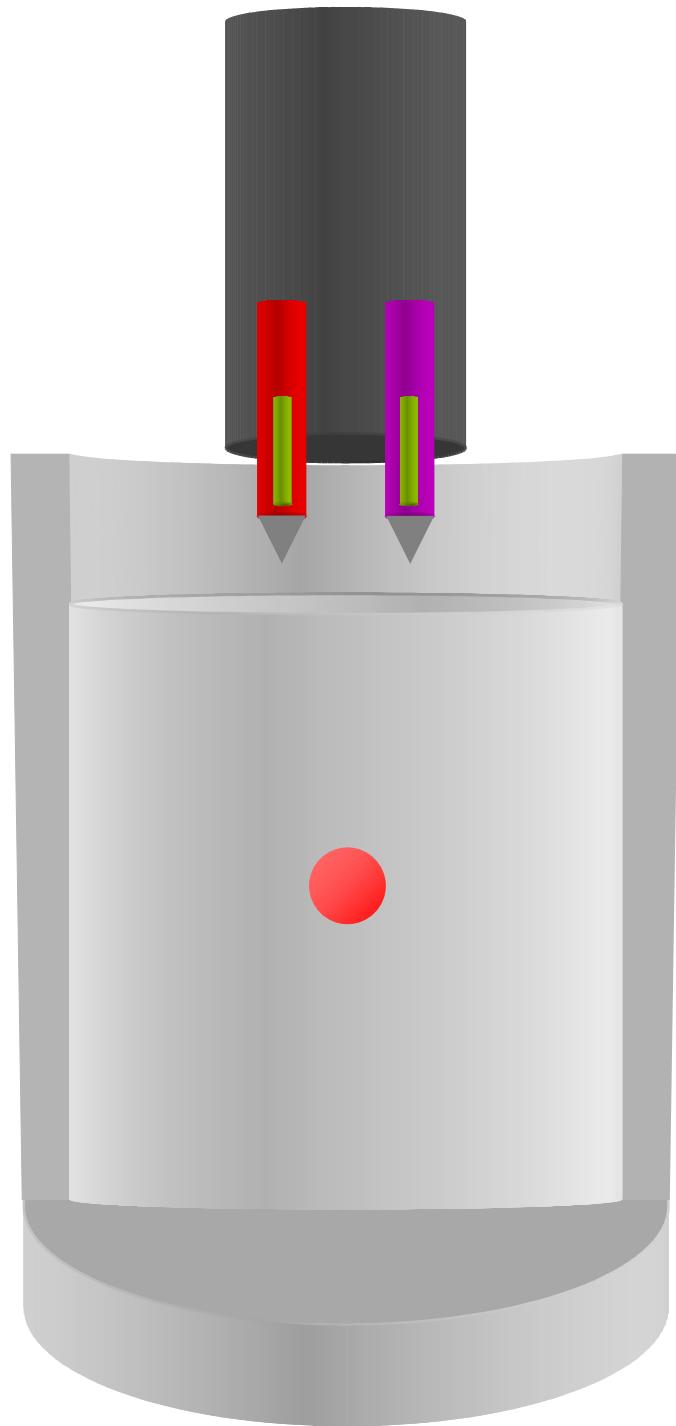
5.5 μm
polystyrene
Microspheres

Or

Magnetic
beads

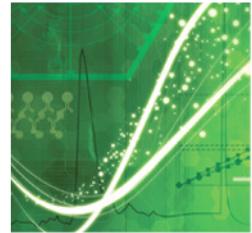




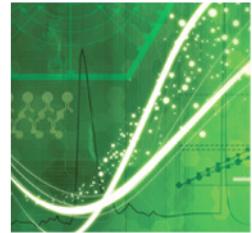




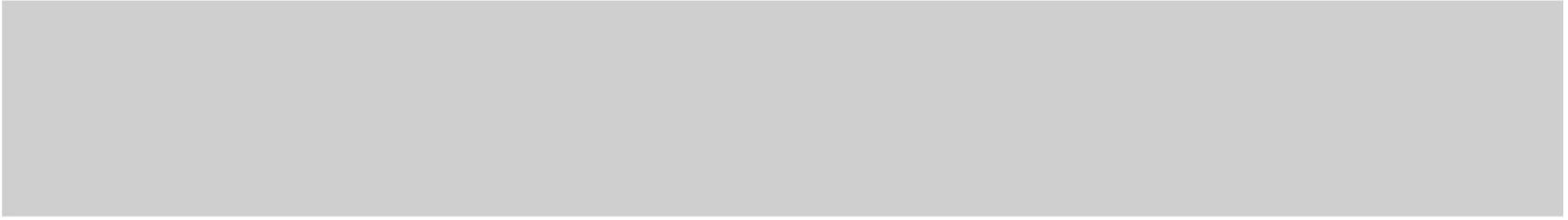
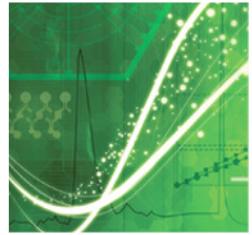
Multiplexing with Colored Bead Sets

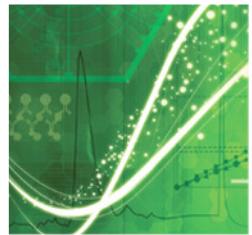


Multiplexing with Colored Bead Sets



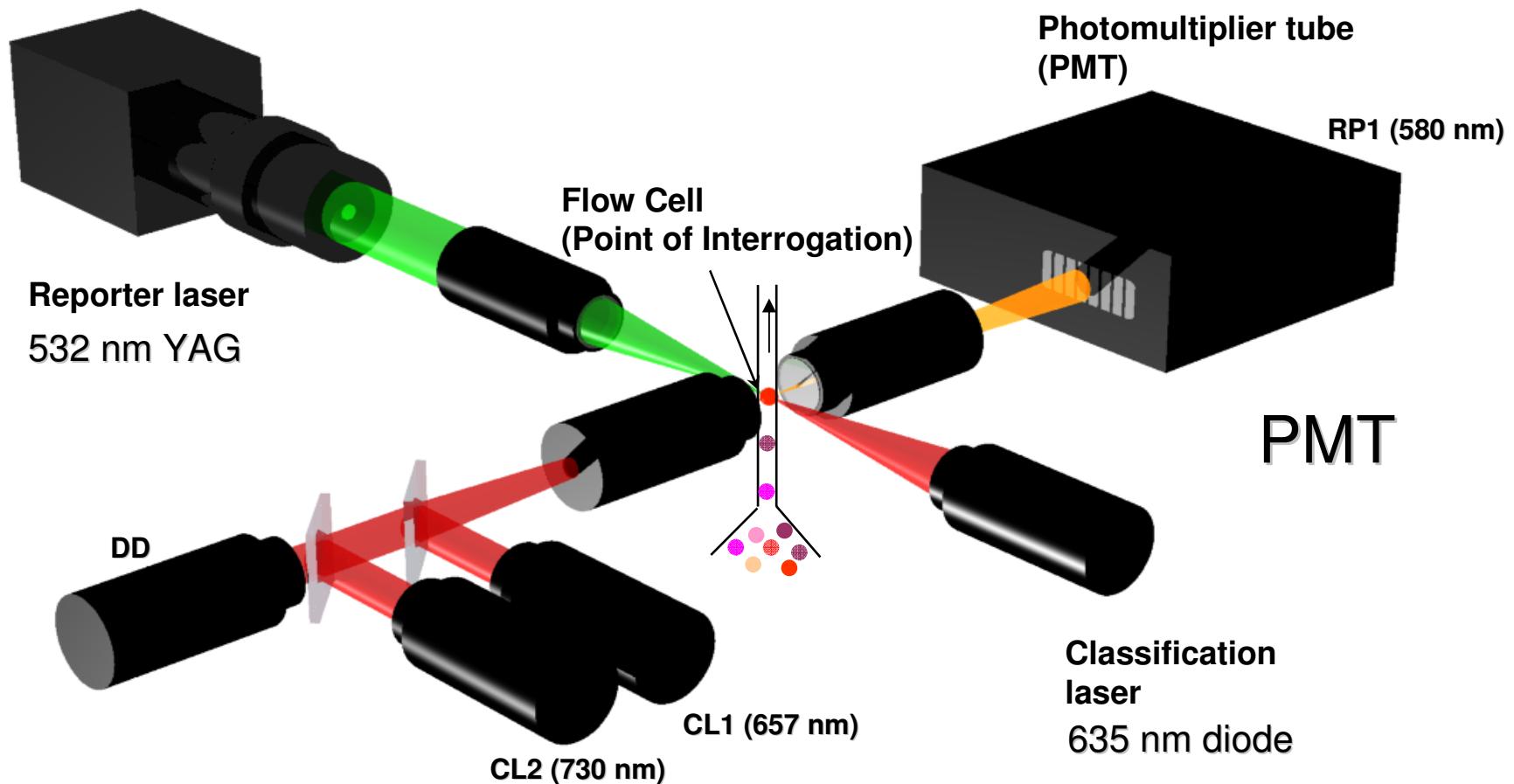
Multiplexing with Colored Bead Sets

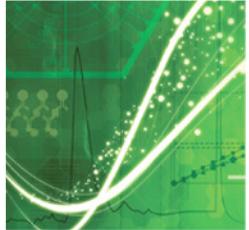






Optical bench





Assay Procedure

Prewet wells & add beads

↓ Wash x2

Add Samples and Standards

↓ Incubate 30' and Wash x3

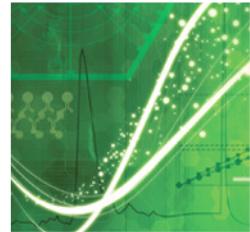
Add Detection Antibody

↓ Incubate 30' and Wash x3

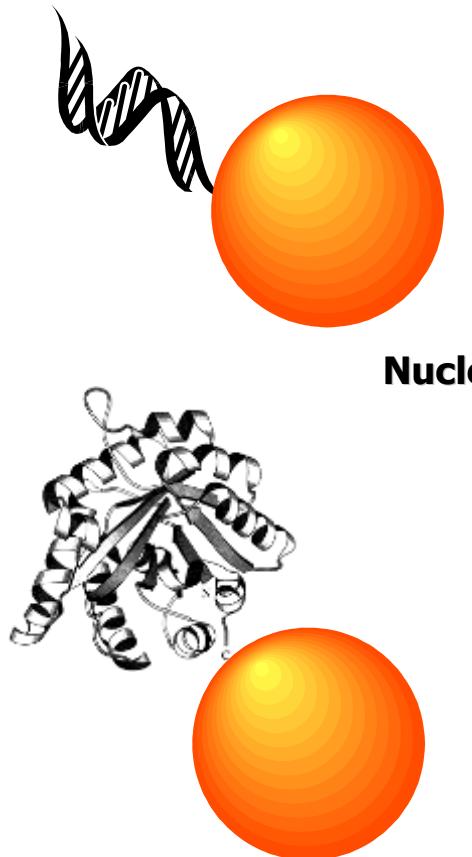
Add Streptavidin-PE

↓ Incubate 10' and Wash x3

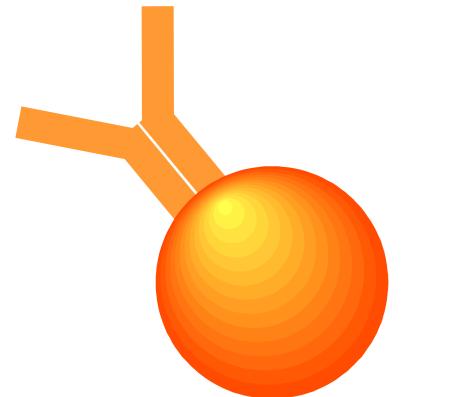
Read Plate



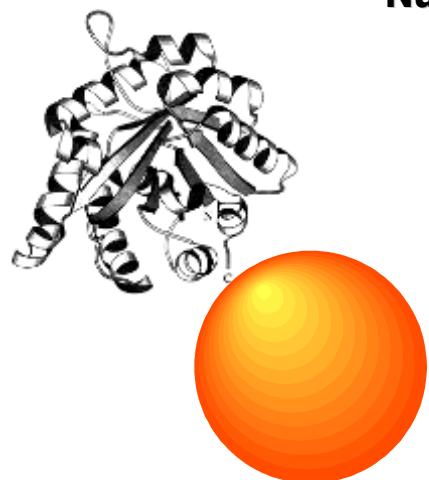
Flexible Platform



Nucleic Acid



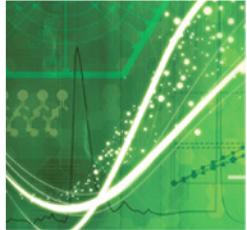
Antibody



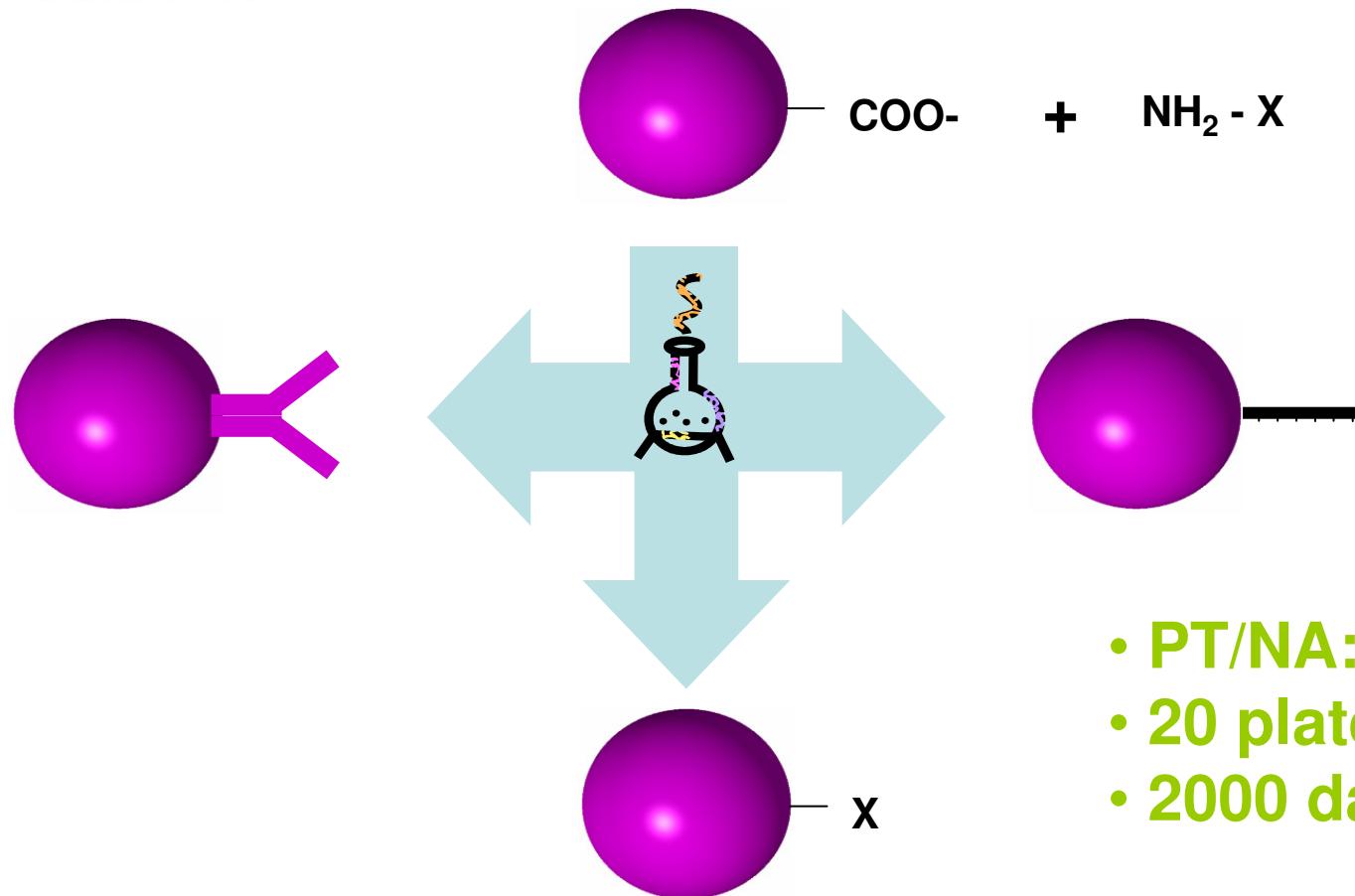
Protein

Your
Molecule
Here

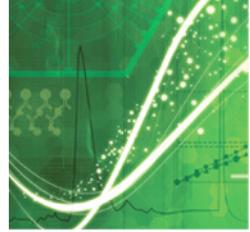




Beads and Coupling Reagents *Homebrew Assays*



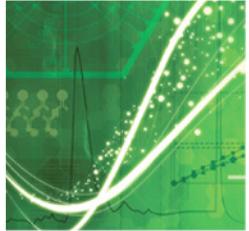
- PT/NA: 5~150KD
- 20 plates
- 2000 data points



The Bio-Plex Solution



www.bio-rad.com/bioplex

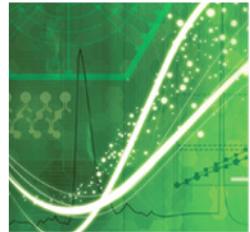


Break through topics

✓ **Multiplex**

✓ **High throughput**

✓ **Automatics**

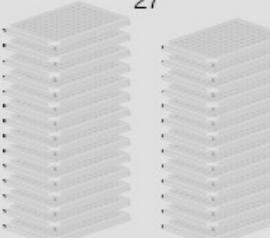


Multiplexing Increases Throughput

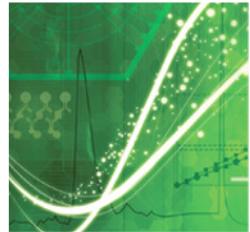
How would you like to have 2,160 data points in one 96-well plate?

The Power of the Bio-Plex System

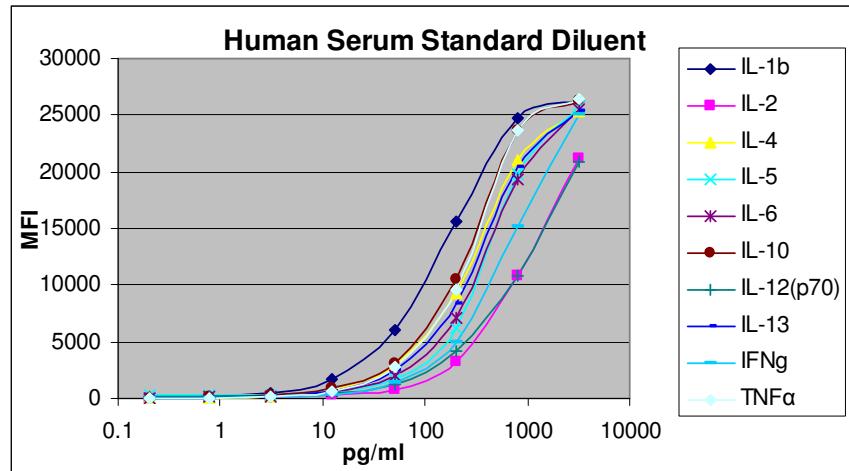
Side-by-Side Comparison: Analyzing 27 Cytokines in 80 Samples

	ELISA	Bio-Plex
Number of cytokines	27	27
Number of samples	80	80
Total data points	2,160	2,160
Number of 96-well plates	27 	1 
Data points per plate	80	2,160
Total time required	>60 hr	3 hr
Sample volume	Serum or plasma, >1 ml* Cell culture supernatant, >1 ml*	Serum or plasma, 12.5 µl Cell culture supernatant, 50 µl
Assay range	Serum or plasma, 2–3,000 pg/ml Cell culture supernatant, 2–3,000 pg/ml	Serum or plasma, ~0.2–3,200 pg/ml Cell culture supernatant, ~2–32,000 pg/ml

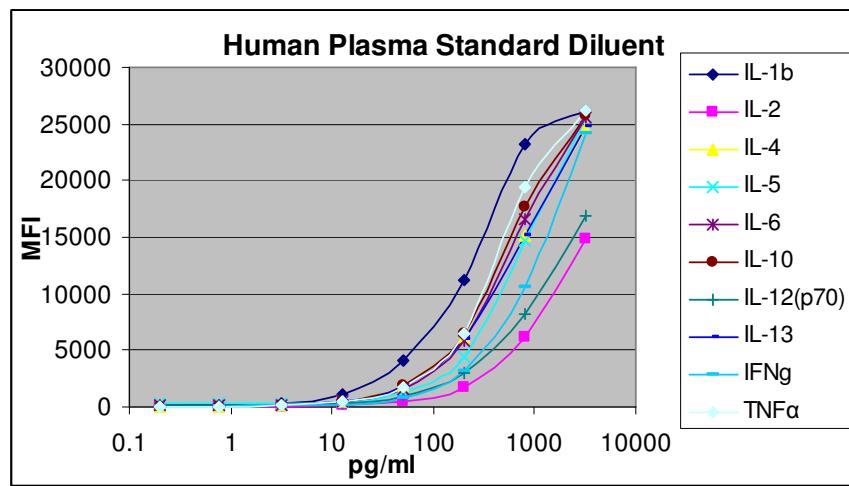
* Based on 50 µl/well of sample.

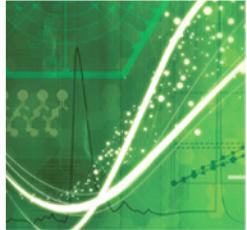


Parallelism in Serum and Plasma



Standard curves in serum and plasma





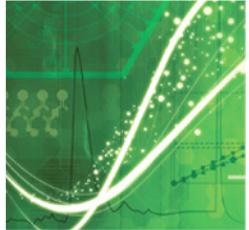
Who should need this?

Life Science Research

- Extracellular signaling proteins
- Intracellular signaling proteins
- Cancer markers
- Cardiac markers
- Endocrine
- Isotyping
- Metabolic Markers
- Neurobiology
- Transcription Factors
- Gene Expression
- Genotyping
- MicroRNA profiling

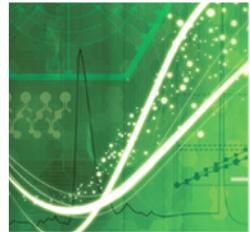
Diagnostics

- Genetic Disease
- Allergy Testing
- Autoimmune Disease
- HLA Testing
- Infectious Disease
- Biodefense



Industrial Bio-Plex Placements — A Sampling

- Abbott
- Altana Pharma
- Amgen
- AstraZeneca
- Bayer
- Biogen Idec
- Bristol-Myers Squibb
- Centocor (J&J)
- Charles River Labs (CRL)
- Critical Therapeutics
- Eli Lilly
- Enzon Pharmaceuticals
- Genzyme
- GE Healthcare
- GlaxoSmithKline (GSK)
- Institute for Bioanalytics (IBA)
- IBT Reference Labs
- Jackson Laboratories
- Linco Diagnostic Services
- Merck
- Proctor & Gamble
- Pfizer
- Quest Pharmaceutical Services
- R.J. Reynolds
- Ross Products
- Sanofi-Aventis
- Schering-Plough
- Synta Pharmaceuticals
- Therakos (J&J)
- TGA Sciences
- Upstate Biotechnology
- Wyeth



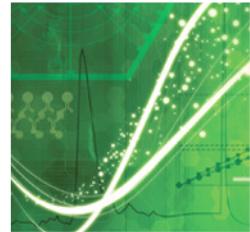
Our Partners in Taiwan

- 行政院衛生署疾病管制局 x7
- 中央研究院 生醫所 x1
- 國家衛生研究院 精神醫學組 x1
- 核能研究所 同位素研究組 x1
- 淡水家畜試驗中心 豬瘟組 x1
- 國防醫學院 麻醉學科 x1
- 長庚醫院 檢驗中心 x1
- 長庚大學 蛋白質體中心 x1
- 台大醫學院 藥理所 x1
- 台灣大學 植病系 x1
- 國泰醫院汐止分院 醫學研究中心 x1
- 和信醫院 治癌中心 x1
- 中山大學 生科院 x1
- 彰化基督教醫院 檢驗科 x1
- 冷泉港生物科技公司 x1

Total : 21

Bio-Plex Technology in Protein application





Bio-Plex Assays

A Broad Menu of Cytokine Targets - Human

Human Cytokine Assays

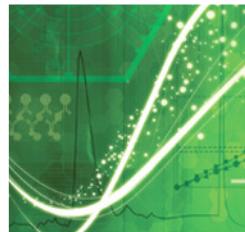
Group I	8-Plex A Panel (171-A11080)	9-Plex B Panel (171-A11100)	Th1/Th2 Panel (171-A11081)	17-Plex Panel (171-A11171)	27-Plex Panel (171-A11127)	Available x-Plex Targets*
IL-1 β		●		●	●	●
IL-1ra					●	●
IL-2	●		●	●	●	●
IL-4	●		●	●	●	●
IL-5		●	●	●	●	●
IL-6	●			●	●	●
IL-7		●		●	●	●
IL-8	●			●	●	●
IL-9					●	●
IL-10	●		●	●	●	●
IL-12 (p70)		●	●	●	●	●
IL-13		●	●	●	●	●
IL-15					●	●
IL-17		●		●	●	●
Basic FGF					●	●
Eotaxin					●	●
G-CSF		●		●	●	●
GM-CSF	●		●	●	●	●
IFN- γ	●		●	●	●	●
IP-10					●	●
MCP-1 (MCAF)		●		●	●	●
MIP-1 α					●	●
MIP-1 β		●		●	●	●
PDGF-BB					●	●
RANTES					●	●
TNF- α	●		●	●	●	●
VEGF					●	●

Group II	23-Plex Panel (171-A11123)	Available x-Plex Targets*
IL-1 α	●	●
IL-2R α	●	●
IL-3	●	●
IL-12 (p40)	●	●
IL-16	●	●
IL-18**	●	●
CTACK	●	●
GRO- α	●	●
HGF	●	●
ICAM-1***	●	●
IFN- α 2	●	●
LIF	●	●
MCP-3	●	●
M-CSF	●	●
MIF	●	●
MIG	●	●
β -NGF	●	●
SCF	●	●
SOGF- β	●	●
SDF-1 α	●	●
TNF- β	●	●
TRAIL	●	●
VCAM-1***	●	●

* You can design an x-Plex assay with combinations of assays, including custom singleplex assays. For more information, go to www.bio-rad.com/bio-plex/x-plex/ or contact your local Bio-Rad sales representative.

** IL-18 is not available as a singleplex assay, only as part of an assay panel.

*** This assay requires additional sample dilution.



Bio-Plex Assays

A Broad Menu of Cytokine Targets – Mouse and Rat

Mouse and Rat Cytokine Assays

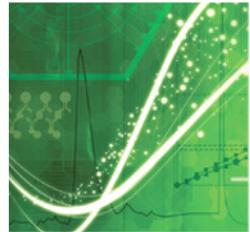
Mouse Group I	8-Plex A Panel (171-F11080)	10-Plex B Panel (171-F11100)	Th1/Th2 Panel (171-F11081)	23-Plex Panel (171-F11171)	Available x-Plex Targets*
IL-1 α		●		●	●
IL-1 β	●			●	●
IL-2	●		●	●	●
IL-3		●		●	●
IL-4	●		●	●	●
IL-5	●		●	●	●
IL-6		●		●	●
IL-9				●	●
IL-10	●		●	●	●
IL-12 (p40)		●		●	●
IL-12 (p70)		●	●	●	●
IL-13				●	●
IL-17		●		●	●
Eotaxin				●	●
G-CSF		●		●	●
GM-CSF	●		●	●	●
IFN- γ	●		●	●	●
KC		●		●	●
MCP-1 (MCAF)				●	●
MIP- α		●		●	●
MIP-1 β				●	●
RANTES		●		●	●
TNF- α	●		●	●	●

* You can design an x-Plex assay with combinations of assays, including custom singleplex assays. For more information, go to www.bio-rad.com/bio-plex/x-plex/ or contact your local Bio-Rad sales representative.

** IL-18 is not available as a singleplex assay, only as part of an assay panel.

Mouse Group II	9-Plex Panel (171-F11009)	Available x-Plex Targets*
IL-15	●	●
IL-18**	●	●
Basic FGF	●	●
LIF	●	●
M-CSF	●	●
MIG	●	●
MIP-2	●	●
PDGF-BB	●	●
VEGF	●	●

Rat	9-Plex A Panel (171-K11070)	Available x-Plex Targets*
IL-1 α	●	●
IL-1 β	●	●
IL-2	●	●
IL-4	●	●
IL-6	●	●
IL-10	●	●
GM-CSF	●	●
IFN- γ	●	●
TNF- α	●	●



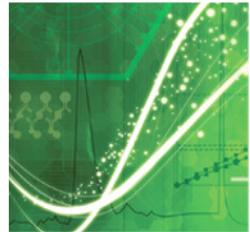
Bio-Plex Cytokine Assays

Assays	Human	Mouse	Rat
IL-1 α	● new	●	●
IL-1 β	●	●	●
IL-1ra	●		
IL-2	●	●	●
IL-2R α	● new		
IL-3	● new	●	
IL-4	●	●	●
IL-5	●	●	
IL-6	●	●	●
IL-7	●		
IL-8	●		
IL-9	●	●	
IL-10	●	●	●
IL-12 (p40)	● new	●	
IL-12 (p70)	●	●	
IL-13	●	●	
IL-15	●	● new	
IL-16	● new		
IL-17	●	●	
IL-18**	● new	● new	
Basic FGF	●	● new	
CTACK	● new		
Eotaxin	●	●	
G-CSF	●	●	
GM-CSF	●	●	●
GRO- α	● new		

Assays	Human	Mouse	Rat
HGF	● new		
ICAM-1	● new		
IFN- α 2	● new		
IFN- γ	●	●	●
IP-10	●		
KC			●
LIF	● new	● new	
MCP-1 (MCAF)	●	●	
MCP-3	● new		
M-CSF	● new	● new	
MIF	● new		
MIG	● new	● new	
MIP-1 α	●	●	
MIP-1 β	●	●	
MIP-2		● new	
β -NGF	● new		
PDGF-BB	●	● new	
RANTES	●	●	
SCF	● new		
SCGF- β	● new		
SDF-1 α	● new		
TNF- α	●	●	●
TNF- β	● new		
TRAIL	● new		
VCAM-1	● new		
VEGF	●	● new	

An Expanding Collection of Assays

- 50 Human
- 32 Mouse
- 9 Rat



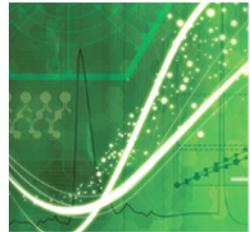
Bio-Plex Precision Pro Working Assay Ranges

Representative Working Assay Range*

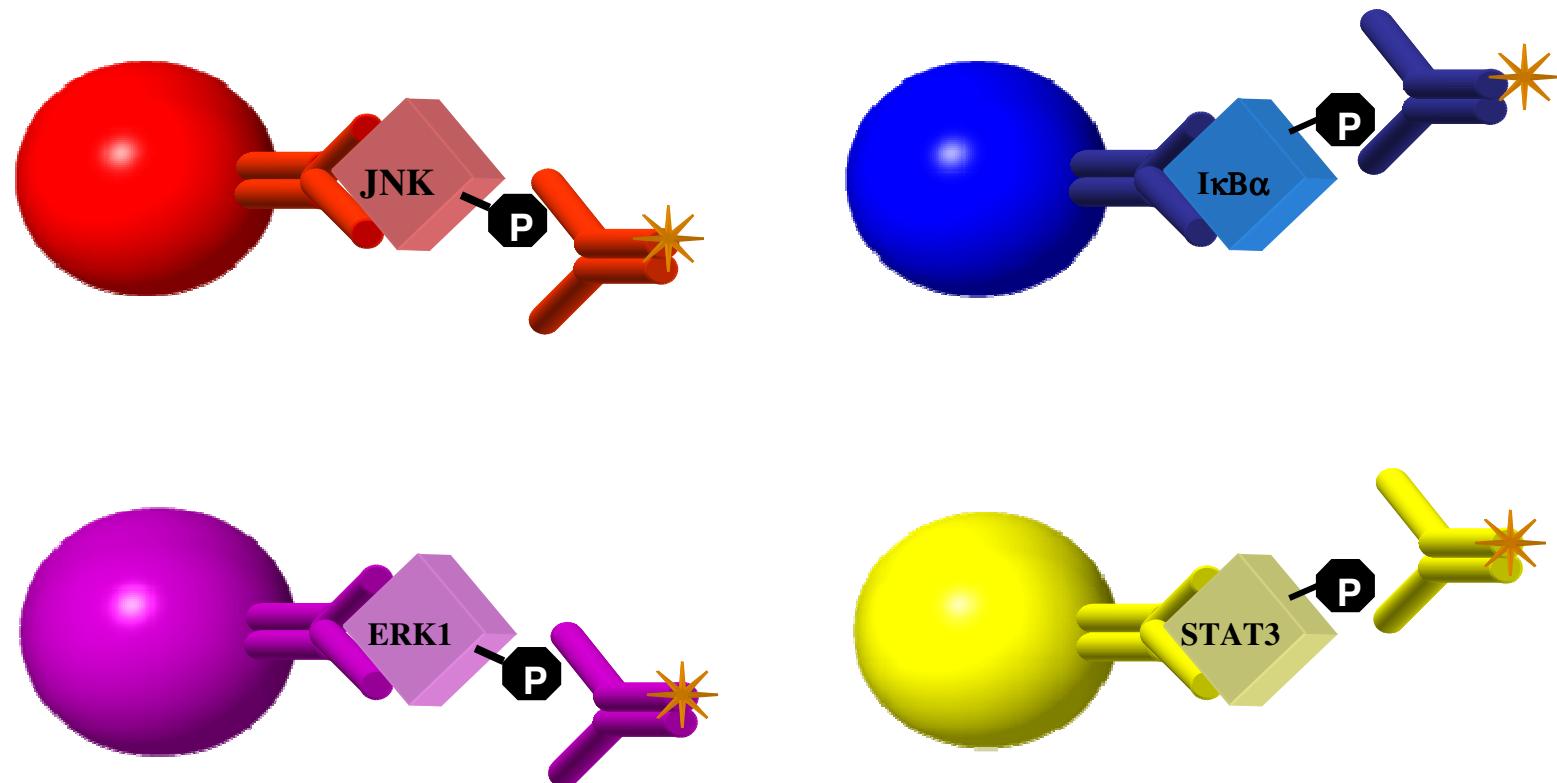
Analyte	Serum	Plasma
IL-1 β	0.2–520	0.2–560
IL-2	1.9–3,200	3.13–3,200
IL-4	0.4–1,707	0.3–3,200
IL-5	2.7–3,200	3.7–3,200
IL-6	0.3–2,760	0.9–3,200
IL-10	0.2–2,320	0.3–3,200
IL-12 (p70)	0.5–3,200	1.4–3,200
IL-13	0.2–2,320	0.4–3,200
IFN- γ	0.9–3,200	1.2–3,200
TNF- α	0.2–1,880	0.2–2,720

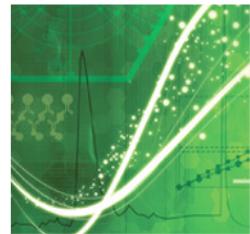
* Results (in pg/ml) based on an average of 5 assays meeting specifications for precision and accuracy. For both serum and plasma samples, intra-assay CV was $\leq 8\%$ and inter-assay CV was $\leq 10\%$.

- High sensitivity
- High precision
- 3 hrs to results



Bio-Plex Phosphoprotein Assays (Signal Transduction Assays)





Bio-Plex Assays

A Broad Menu of Signal Transduction Assays

Available Assays

Assays	Phosphoprotein	Total
Akt Signaling		
Akt (Ser ⁴⁷³)	●	●
GSK-3α/β (Ser ²¹ /Ser ⁹)	●	
Cell Cycle/Checkpoint Control		
p53 (Ser ¹⁵)	●	●
p53 (Ser ³⁷)	●	●
p53 (Ser ⁴⁶)	●	●
Chromatin Regulation/Acetylation		
Histone H3 (Ser ¹⁰)	●	
Immunology/Inflammation		
IκB-α (Ser ³² /Ser ³⁶)	●	●
NF-κB p65 (Ser ⁵³⁶)	●	
STAT2 (Tyr ⁶⁸⁹)	●	
STAT3 (Ser ⁷²⁷)	●	
STAT3 (Tyr ⁷⁰⁵)	●	
STAT6 (Tyr ⁶⁴¹)	●	
Tyk2 (Tyr ¹⁰⁵⁴ /Tyr ¹⁰⁵⁵)	●	
Glucose/Energy Metabolism		
IGF-IR (Tyr ¹¹³¹)	●	
IR-β (Tyr ¹¹⁴⁶)	●	
IRS-1 (Ser ⁶³⁶ /Ser ⁶³⁹)	●	

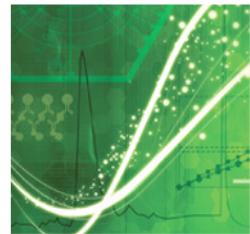


CST antibodies exclusively developed and validated for Bio-Plex phosphoprotein and total target assays.

Unique

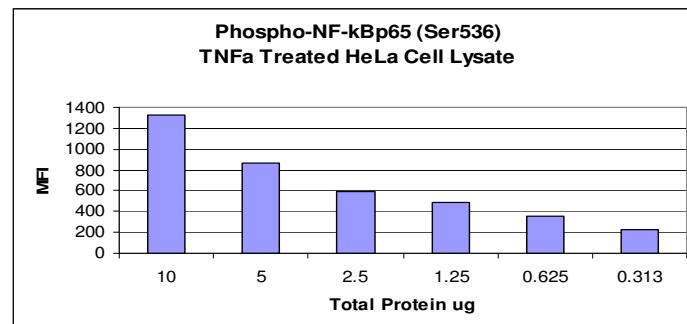
Phosphoproteins : 36
Total Proteins : 16

Assays	Phosphoprotein	Total
MAP Kinase Signalling		
ATF-2 (Thr ⁷¹)	●	●
c-Jun (Ser ⁸³)	●	●
ERK1 (Thr ²⁰² /Tyr ²⁰⁴)	●	
ERK2 (Thr ¹⁸⁵ /Tyr ¹⁸⁷)	●	●
ERK1/2 (Thr ²⁰² /Tyr ²⁰⁴ , Thr ¹⁸⁵ /Tyr ¹⁸⁷)	●	●
HSP27 (Ser ⁷⁸)	●	●
JNK (Thr ¹⁸³ /Tyr ¹⁸⁵)	●	●
MEK1 (Ser ²¹⁷ /Ser ²²¹)	●	●
p38 MAPK (Thr ¹⁸⁰ /Tyr ¹⁸²)	●	●
p90RSK (Thr ³⁵⁹ /Ser ³⁶³)	●	●
Neuroscience		
CREB (Ser ¹³³)	●	●
TrkB (Tyr ⁴⁹⁰)	●	
Translational Control		
p70 S6 kinase (Thr ⁴²¹ /Ser ⁴²⁴)	●	
S6 ribosomal protein (Ser ²³⁵ /Ser ²³⁶)	●	
Tyrosine Kinases		
Bcr-Abl (Tyr ²⁴⁵)	●	●
c-Abl (Tyr ²⁴⁵)	●	
c-Abl (Tyr ⁴¹²)	●	
EGFR (Tyr)	●	
PDGF receptor-β (Tyr ⁷⁵¹)	●	
Src (Tyr ⁴¹⁶)	●	

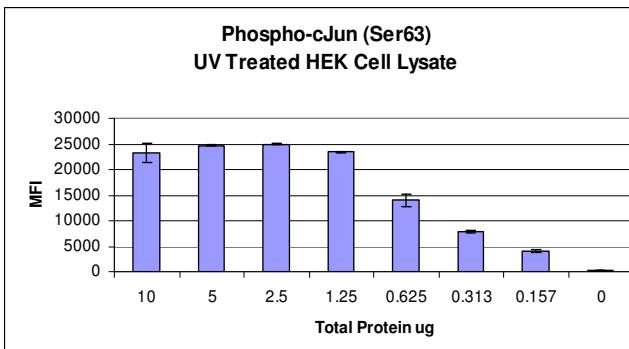


Superior Sensitivity and Range

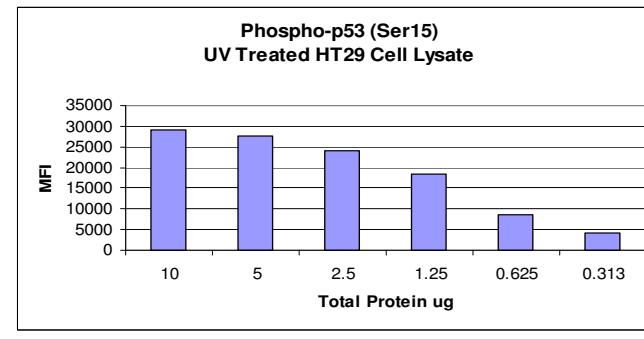
Phospho NF kB p65



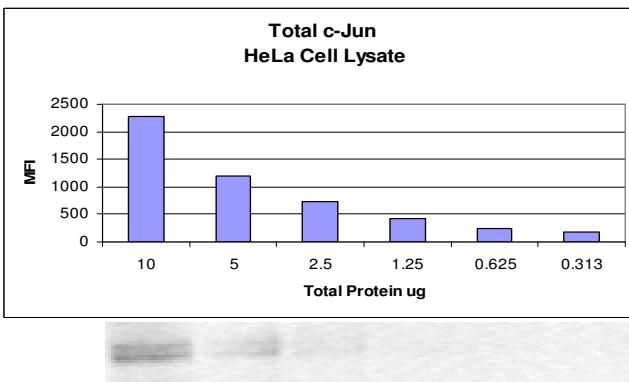
Phospho c-Jun

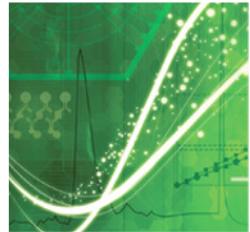


Phospho p53



Total c-Jun





Bio-Plex Pro Assays

Assays Focused on Disease States

Bio-Plex Pro Disease Research Assays

- On 8 micron magnetic beads
- Currently fixed panels
- Mouse Diabetes (Coming Soon)

Available Targets

Human Acute Phase	Human Angiogenesis	Diabetes		Human Isotyping	Precision Pro™ Human Cytokines
		Human	Mouse		
α -2-macroglobulin	Angiopoietin-2	Adiponectin	Adiponectin	IgG ₁	IL-1 β
CRP	Follistatin	Adipsin	—	IgG ₂	IL-2
Ferritin	G-CSF	C-peptide	—	IgG ₃	IL-4
Fibrinogen	HGF	Ghrelin	Ghrelin	gG ₄	IL-5
Haptoglobin	IL-8	GIP	GIP	IgM	IL-6
Procalcitonin	Leptin	GLP-1	GLP-1	IgA	IL-10
SAA	PDGF-BB	Glucagon	Glucagon	IgE	IL-12 (p70)
SAP	PECAM-1	IL-6	IL-6		IL-13
Tissue plasminogen activator	VEGF	Insulin	Insulin		IFN- γ
		Leptin	Leptin		TNF- α
		PAI-1	PAI-1		
		Resistin	Resistin		
		TNF- α	—		
		Vlsfatin			

Coming soon

Welcome to **Bio-Rad Laboratories**

please | register | close
log in | today | window |

x-Plex Ordering System

Bio-Plex x-Plex Assays

Assay Group and Package Size

Human Cytokines

- IL-1beta IL-7 IL-15
- IL-1ra IL-8 IL-17
- IL-2 IL-9 IP-10
- IL-4 IL-10 Eotaxin
- IL-5 IL-12 (p70) FGF Bas
- IL-6 IL-13 G-CSF

Step 1. Select the "Assay Group" and "Package Size".
Step 2. Select the assays that will be mixed to create this x-Plex assay. For cytokine assays, select either ERK1 or ERK2 or ERK1/2. The ATF-2 and c-Jun assays are not multiplexable. The ATF-2 and c-Jun assay cannot be multiplexed together.
Step 3. Select the "View Catalog #" to view the unique catalog number for this x-Plex assay.
NOTE: Follow steps 4 and 5 (next screen) for this unique catalog number to be activated in Bio-Rad's ordering system. Not completing steps 4 and 5 may delay order processing with Customer Service.

x-Plex Assay
Catalog # **Description**


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Bio-Plex x-Plex Assays

Assay Group and Package Size

Phosphoproteins

- Akt ERK1/2 p53 STAT3
- ATF-2 GSK-3a/B p90RSK TrkA
- c-Jun HSP27 p38 MAPK
- EGFR IKB-a p70 S6 Kinase
- ERK1 JNK S6 Ribosomal Protein
- ERK2 NF-kB p65 STAT2

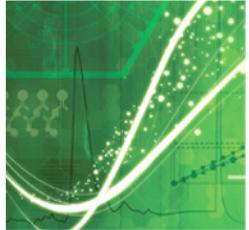
Step 1. Select the "Assay Group" and "Package Size".

Step 2. Select the assays that will be mixed to create this x-Plex assay. For phosphoprotein assays, select either ERK1 or ERK2 or ERK1/2. The EGFR and S6 Ribosomal Protein assay is not multiplexable. The ATF-2 and c-Jun assay cannot be multiplexed together. The IKB-a and NF-kB p65 assay cannot be multiplexed together.

Step 3. Select the "View Catalog #" to view the unique catalog number for this x-Plex assay.

NOTE: Follow steps 4 and 5 (next screen) for this unique catalog number to be activated in Bio-Rad's ordering system. Not completing steps 4 and 5 may delay order processing with Customer Service.

x-Plex Assay
Catalog # **Description**



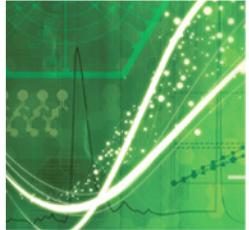
Cytokine Assay samples?

Now >300 Articles

- Human Samples
 - Blister Fluid
 - Bronchoalveolar Lavage Fluid
 - Cerebrospinal Fluid
 - Fat (Adipose) Interstitial Fluid
 - Nasal Lavage Fluid
 - Peritoneal Fluid
 - Plasma*
 - Serum*
 - Synovial Fluid
 - Tissue Culture Supernatant*
- Mouse Samples
 - Plasma*
 - Serum*
 - Bronchoalveolar Lavage Fluid
 - Synovial Fluid/Patellar Washouts
 - Tissue-Colon, Kidney, Lung, Nervous System & Spleen
 - Tissue Culture Supernatant*
- Rat Samples
 - Bronchoalveolar Lavage Fluid
 - Plasma*
 - Serum*
 - Tissue –Colon & Nervous System
 - Tissue Culture Supernatant*

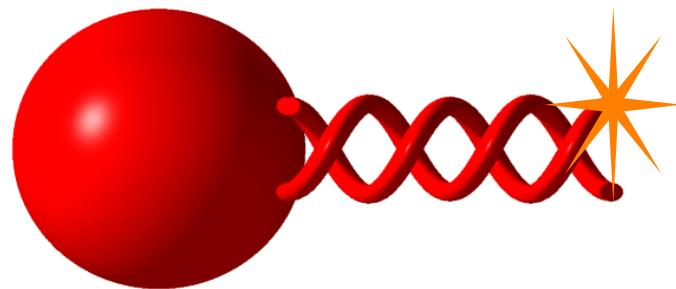
Bio-Plex Technology in Nucleic Acid application



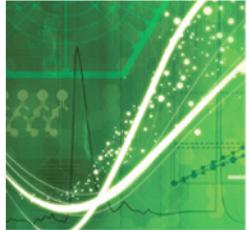


NAT Application

- Pathogen detection, ex Upper respiratory track
- Pathogen Sub typing, ex EV group, R71....
- Gene Expression, ex Tumer marker, genetic disease
- SNP, ex Genetic diseases



Nucleic acid-based assay



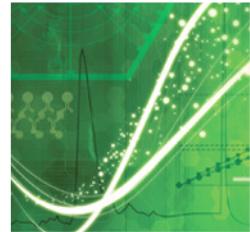
Department of Health and Human Services

Centers for Disease Control and Prevention

Major clinical laboratories e.g. American Centers for Disease Control (CDC) exploit the power of Bio-Plex for research and clinical screening in their 100 response Laboratories

Applications include;

- Syphilis screening (cytokine)
- Dengue virus types 1-4 detection
- West Nile Virus detection
- *Legionella*
- Varicella Zoster Virus (VZV)Ab Assay
- Tuberculosis “spoligotyping” – DNA-based screening
- Bioterrorism agents
- Genaco Acute Respiratory Panel
- Food borne pathogen detection



Nucleic Acid

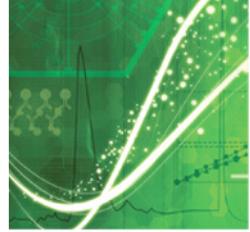
Luminex

Direct Capture of a Labeled PCR Product

Direct Hybridization

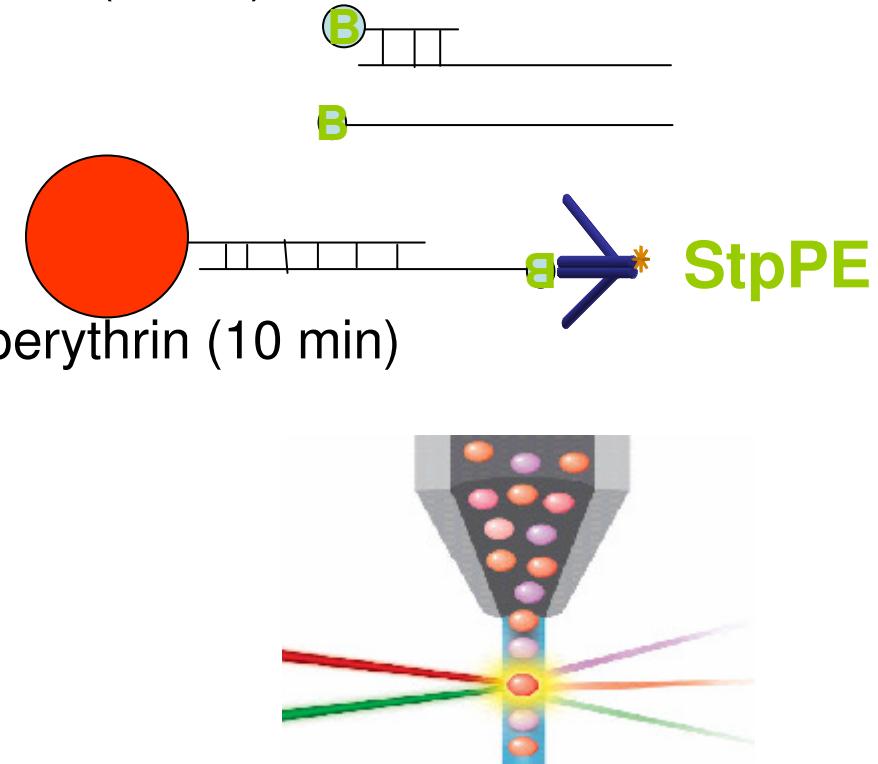
1. Forward Primer – biotin label
2. Reverse Primer
3. Probe for bead – 5' amino modified C12



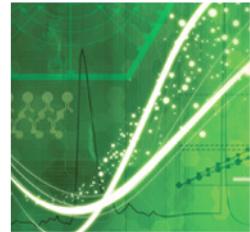


Process

- Multiplex PCR w/ Biotin labeled primer (1.5 hr)
- PCR clean up (0.5 hr)
- Denature (10 min)
- Hybridization (0.5 hr)
- Spin / re-suspend (10 min)
- Hybridization for Streptavidin phycoerythrin (10 min)
- Spin/ re-suspend (10 min)
- Read (30~60 min)



- All in 4 hours for 96 samples and up to 100 targets



Pathogen detection and genotyping

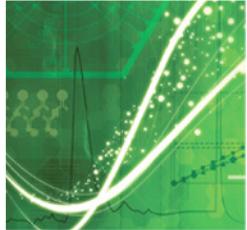
Table 1 PCR primers

Oligo Name	Sequences	Product Sizes
SARS		
SARSF1	GAAGCTATTCTCACGTTCG	
SARSR1	Biotin-CTGTAGAAAATCCTAGCTGGAG	109bp
SARSF2	TTTCTACAGGTTAGCTAACGA	
SARSR2	Biotin-AGCATAAGCAGTTGAGCATC	118bp
SARSF3	TCAACTGCATTGGCAAGCT	
SARSR3	Biotin-CAAGTCGCGAAAGGATATCA	130bp
RSV		
RSVF	GAACAACAGACTACTAGAGA	
RSVR	Biotin-TGTTATAGGCATATCATTGA	124bp
PIV-1		
PIV-1F	TTTCTGGAGATGTCCCGTAGG	
PIV-1R	Biotin-TGAAGGTAGTCAACACATC	121bp
PIV-3		
PIV-3F	AGAGGTCAATACCAACAACTA	
PIV-3R	Biotin-TTGTCTGGTCTGTGACC	111bp
M.Pneumoniae		
MPMF	AAGGACCTGCAAGGGTTCGT	
MPMR	Biotin-GTATGGGCCGTGTCAGTC	138bp
C.Pneumoniae		
CPMF	TGACAACGTAGAAATACAGC	
CPMR	Biotin-GTTCGCTCGTTGCCGGACT	109bp
Enterovirus		
ENTVF	CCTCCGGCCCTGAATGCGG	
ENTVR	Biotin-TAGTCGGTTCGCTGCAGAG	100bp
InfluenzaA		
INFAF	AAGGGCTTCACCGAAGAGG	
INFAR	Biotin-CATTCAAGTCCTCCGATGAG	120bp
InfluenzaB		
INFBF	ATGGCCATCGGATCCTCAAC	
INFBR	Biotin-GTCTCCCTTCTGGTGATA	137bp
Adenovirus		
ADVF	TGGTCAGAGTGCACCAAGCC	
ADVR	Biotin-TTATGTGGTGGCGTTGCCGGC	86bp

Sample	SARSD1	SARSD2	SARSD3	RSVD	PIVID	PIV3D	MPMD	CPMD	ENTD	INFAD	INFBD	ADV D
lum blank	11	10	19	11	13	20	14	14	13	15	17	14
multiplex	1486	1366	280	1717	1316	1049	816	1527	1448	1500	1655	1792
SARS1PC	4450	17	17	16	19	146	13	17	10	15	16	22
SARS2PC	21	4326	17	14	15	177	8	18	16	15	20	12
SARS3PC	16	22	1472	15	21	156	25	68	23	20	11	15
RSVPC	66	14	21	4999	19	128	13	17	11	14	18	10
PIV1PC	17	17	20	18	4229	399	12	17	11	20	19	20
PIV3PC	17	21	19	29	20	3766	22	10	18	25	15	18
MPMPC	17	15	18	15	18	609	3450	18	13	19	29	16
CPMPC	11	11	5	15	15	781	109	4736	12	17	19	9
ENTVPC	17	18	21	44	17	297	15	16	4580	18	17	53
INFAPC	15	14	12	16	13	153	65	14	252	4729	18	26
INFBCP	12	19	16	16	15	335	18	22	12	22	3481	23
ADVPC	12	93	14	20	121	877	18	16	13	21	13	5784

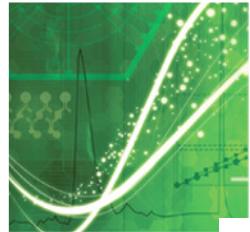
SNP Assays on Bio-Plex





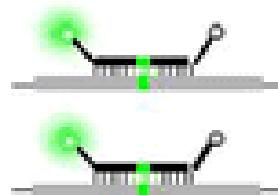
Why use SNPs?

- SNPs (single nucleotide polymorphisms) are genetic markers
 - Over 2 million SNPs in public databases
 - SNPs with a phenotype are a small subset
 - In a gene or regulatory region
 - SNPs that provide genomic region markers are vast majority
 - SNPs are linked to genes/regulatory regions
 - Use as markers for indication of genotype
- Large scale mapping (Single sample)
 - Higher density methods – Chips
- Focused research, screening (Population)
 - Lower density methods – Real-time, Bio-Plex – High throughput

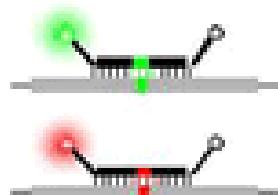


QPCR for SNPs detection -- up to 2 SNP per well

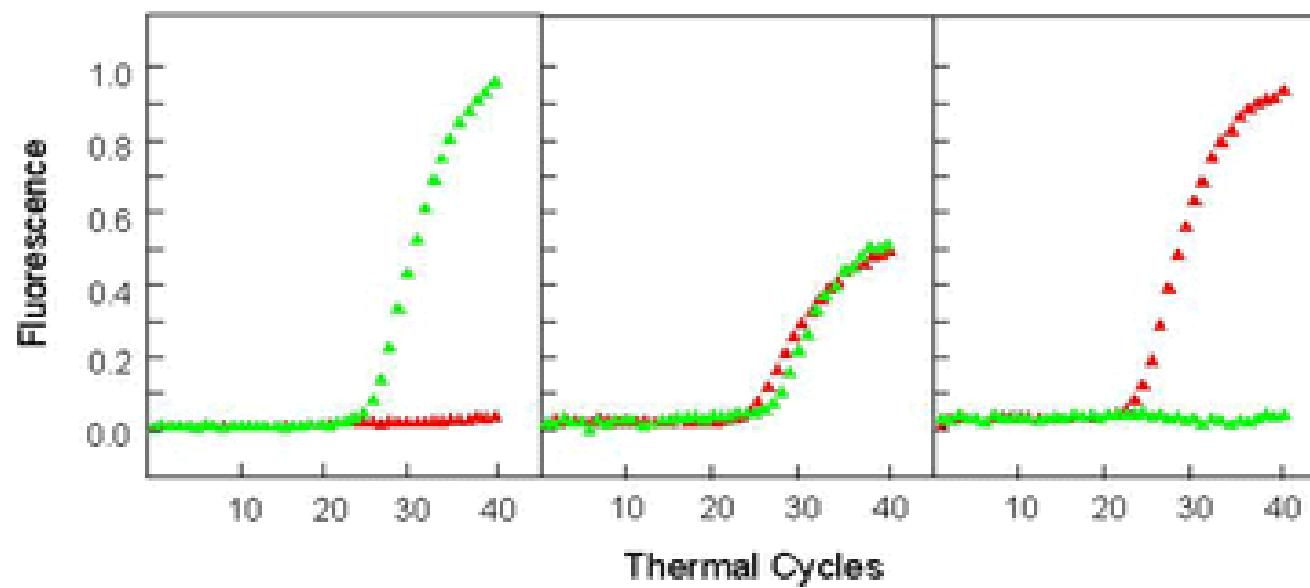
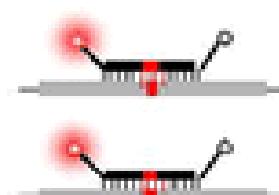
Homozygous Wild-type

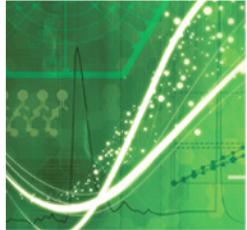


Heterozygote

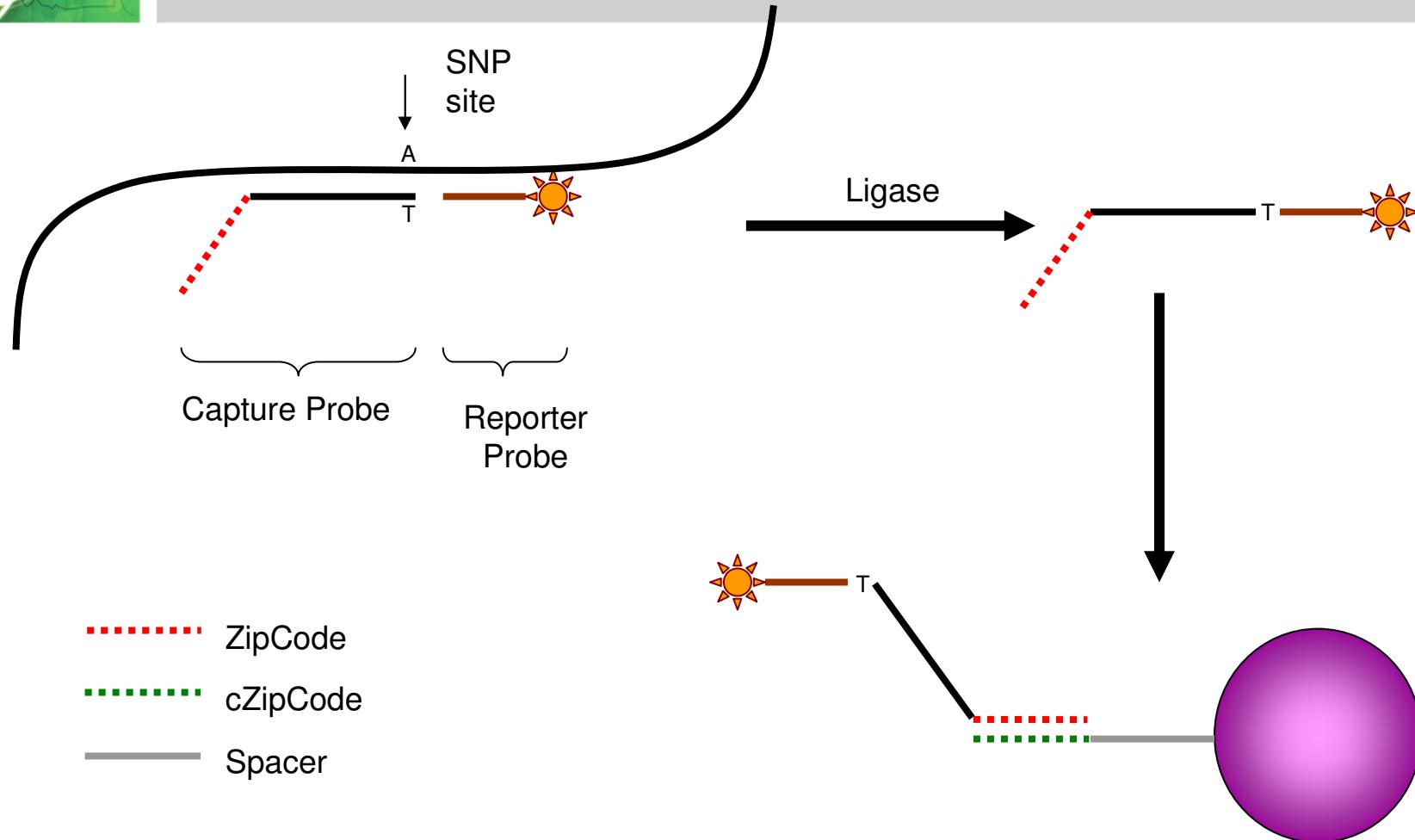


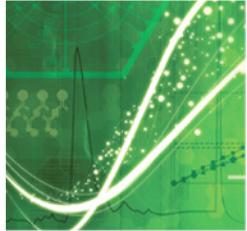
Homozygous Mutant



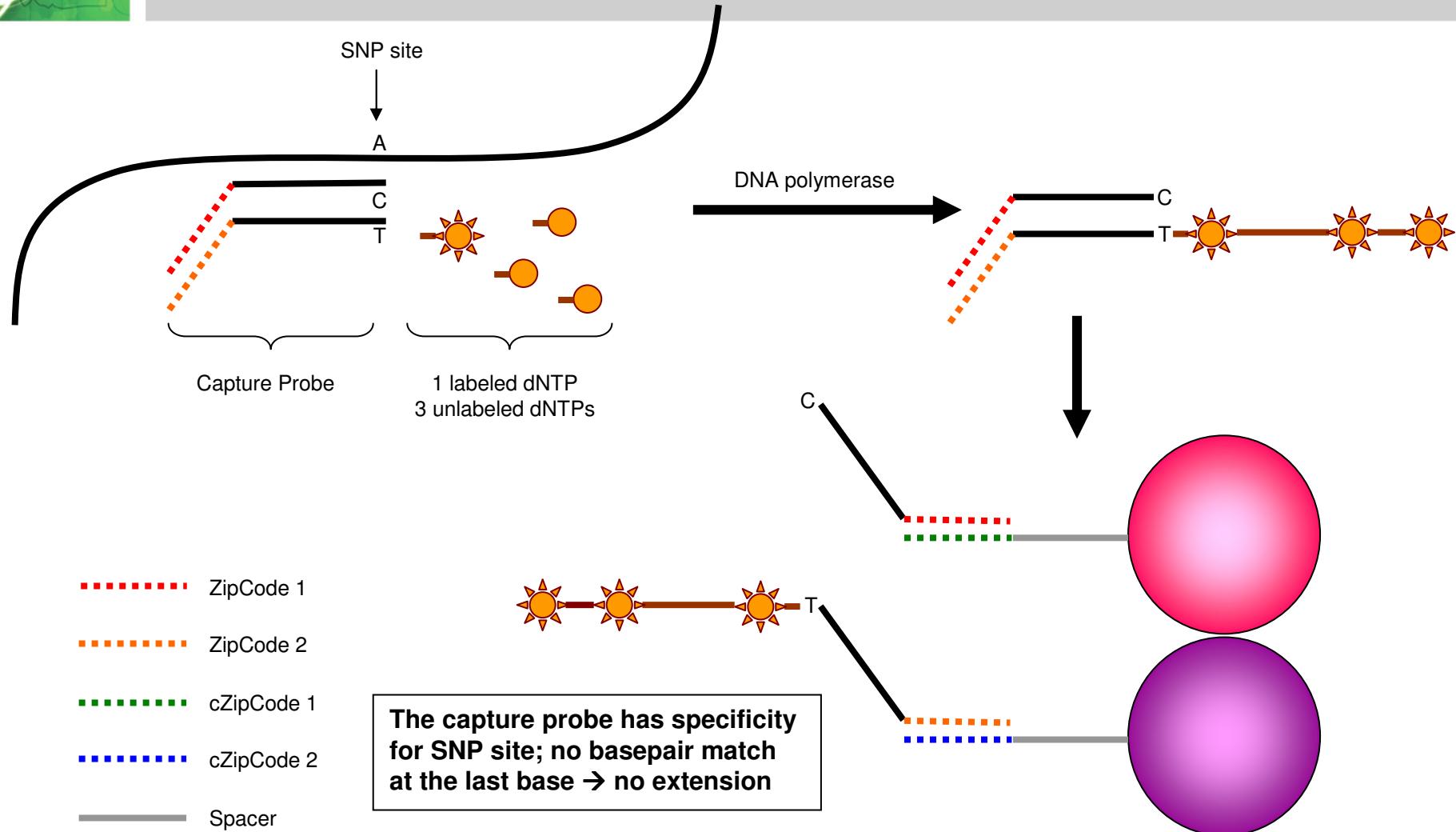


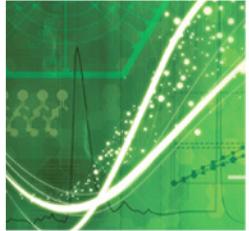
Oligonucleotide Ligation Assay (OLA)





Allele-Specific Primer Extension (ASPE)



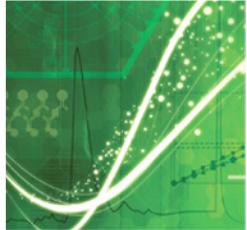


Break through topics

✓ **Multiplex**

✓ **High throughput**

✓ **Automatics**



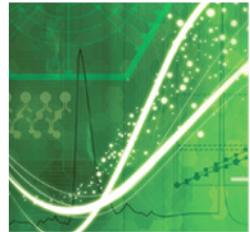
Changing the Customer Experience . . .on washing

From



To





Bio-Plex Pro and Pro II Wash Station

- Two Wash Station Options
 - Bio-Plex Pro Wash Station
 - Magnetic carrier only
 - For Bio-Plex Pro assays
 - Bio-Plex Pro II Wash Station
 - Magnetic and Vacuum carrier
 - For all xMAP assays
- No Programming Required

BIO-RAD

Bio-Plex® Pro
Wash Station

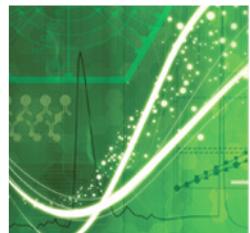


BIO-RAD

Bio-Plex® Pro II
Wash Station

Bio-Plex Pro Wash Station Selection Guide

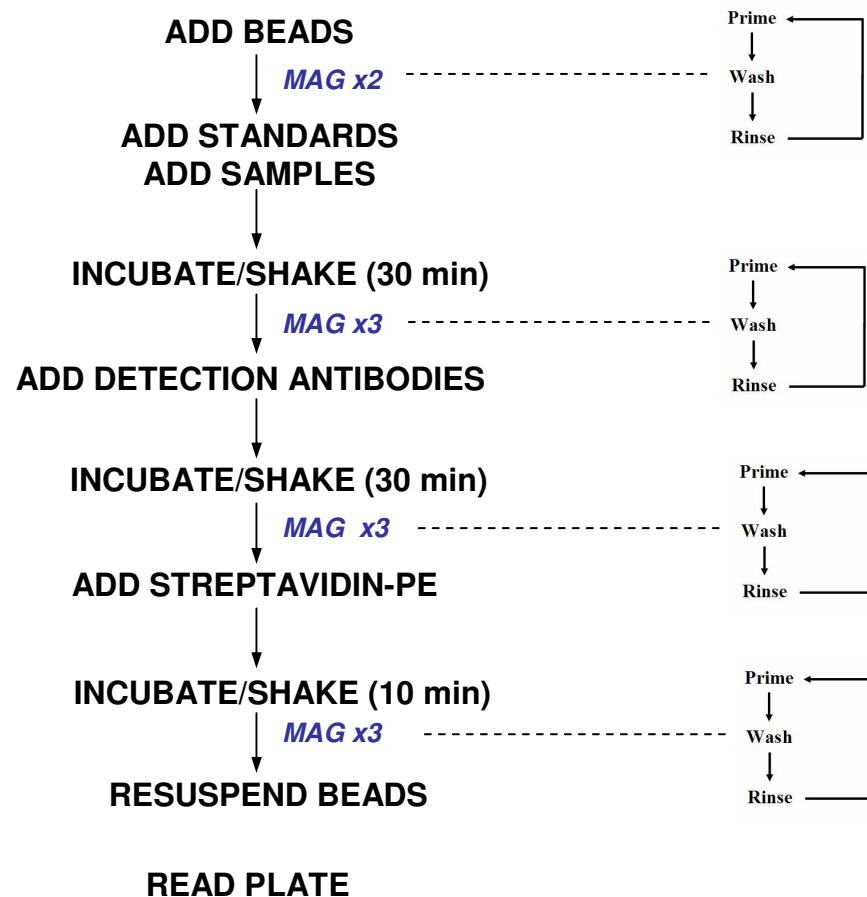
	Bio-Plex Pro Wash Station	Bio-Plex Pro II Wash Station
Bio-Plex assays (polystyrene beads)	—	•
Polystyrene xMAP microspheres	—	•
Bio-Plex Pro assays (magnetic beads)	•	•
Bio-Plex® Precision Pro™ assays (magnetic beads)	•	•
MagPlex microspheres (magnetic beads)	•	•

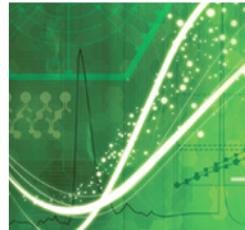


Using Pro Wash Station

BIO-RAD

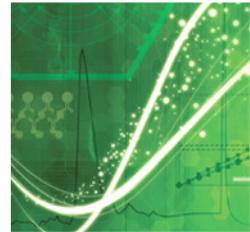
Bio-Plex® Pro
Wash Station





Bio-Plex Pro Wash Station vs. Aurum Vacuum Apparatus

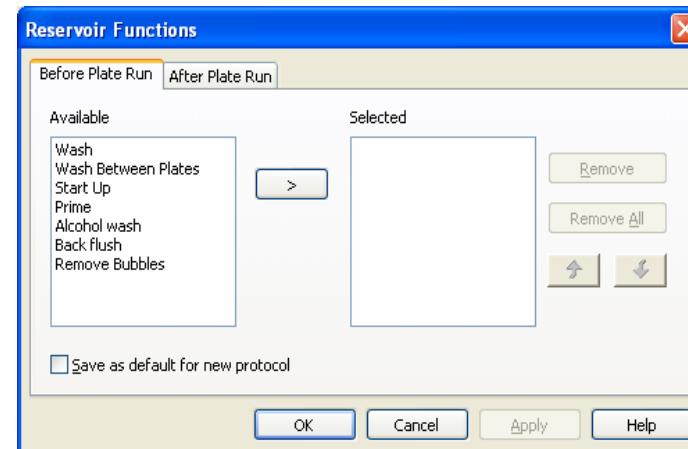
Prewet filter plate	Add beads	Wash	Add standards, controls, and samples, 30 min	Wash	Add detection antibody, 30 min	Wash	Add streptavidin-PE, 10 min	Wash	Read on Bio-Plex suspension array system
→									
 Manual Wash Method					 Automated Wash Method				
<ul style="list-style-type: none">▪ Place plate on vacuum manifold (any bead type), apply and set vacuum strength▪ Filter▪ Remove plate, blot dry base▪ Add wash buffer with multichannel pipet (8 x 12 rows)▪ Filter▪ Remove plate, blot dry base▪ Add wash buffer with multichannel pipet (8 x 12 rows)▪ Filter▪ Remove plate, blot dry base▪ Add wash buffer with multichannel pipet (8 x 12 rows)▪ Filter▪ Remove plate, blot dry base▪ Add next kit reagent▪ Completion time varies per user					<ul style="list-style-type: none">▪ Place plate on Bio-Plex Pro wash station▪ Select Bio-Plex Pro wash program, press start▪ Add next kit reagent▪ Completion time is 3 to 4 min				



Bio-Plex Reservoir

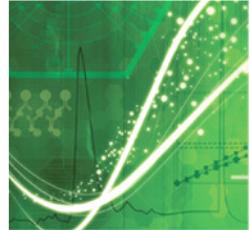
Automated maintenance/walk-away” feature setting up and automatically running unattended “pre” or “post” run washes

Benefit: This feature permits users to use their time more productively,

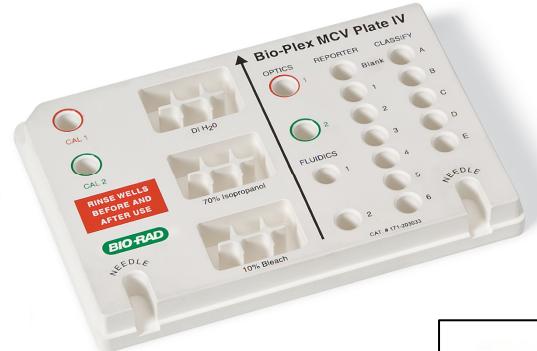


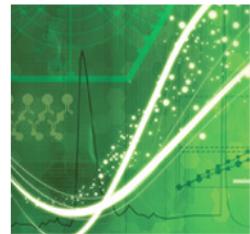
Bio-Plex Manager software





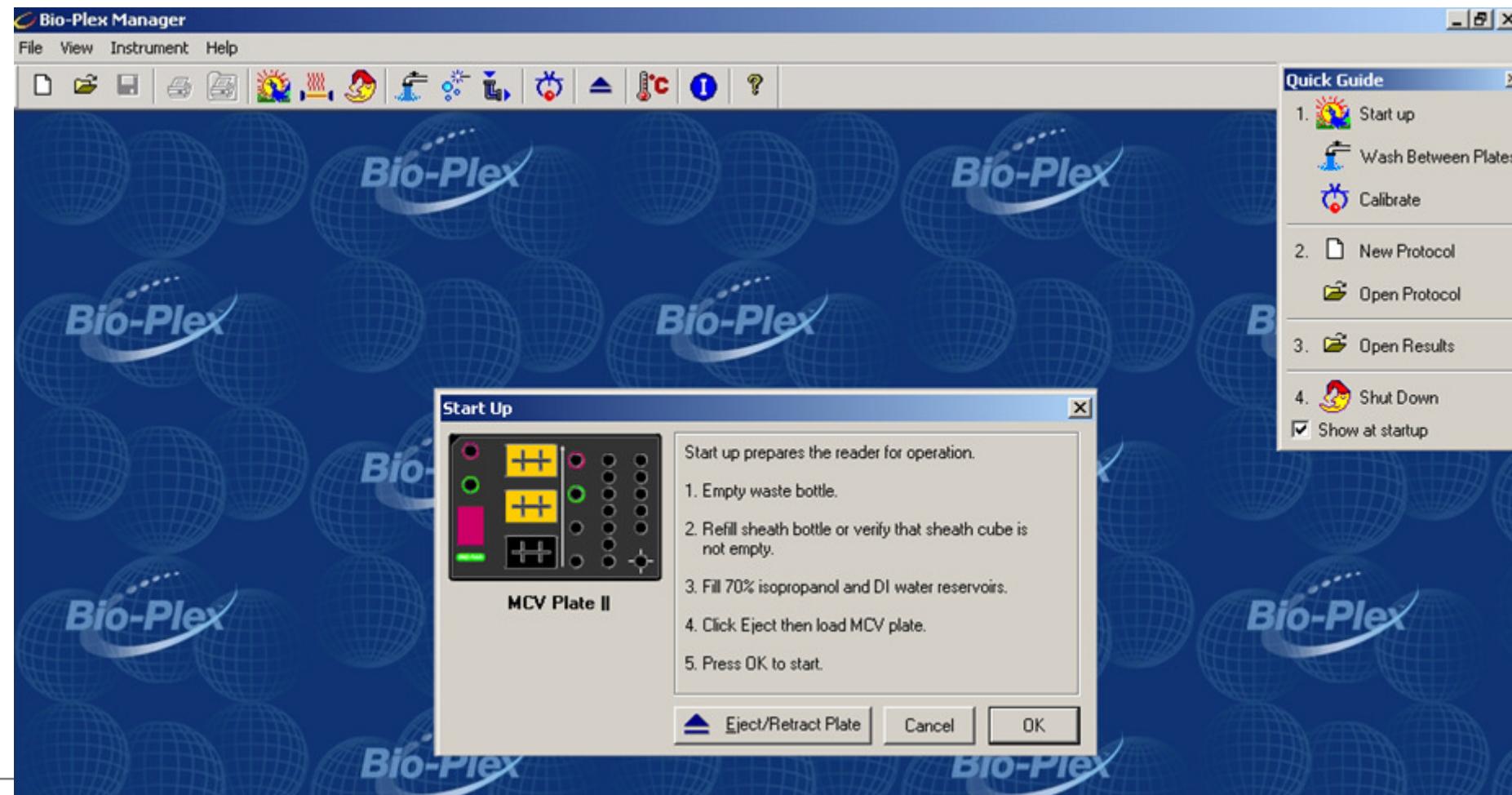
Bio-Plex Manager v 5.0

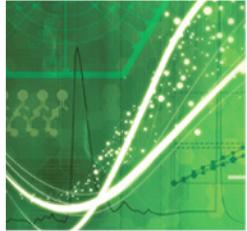




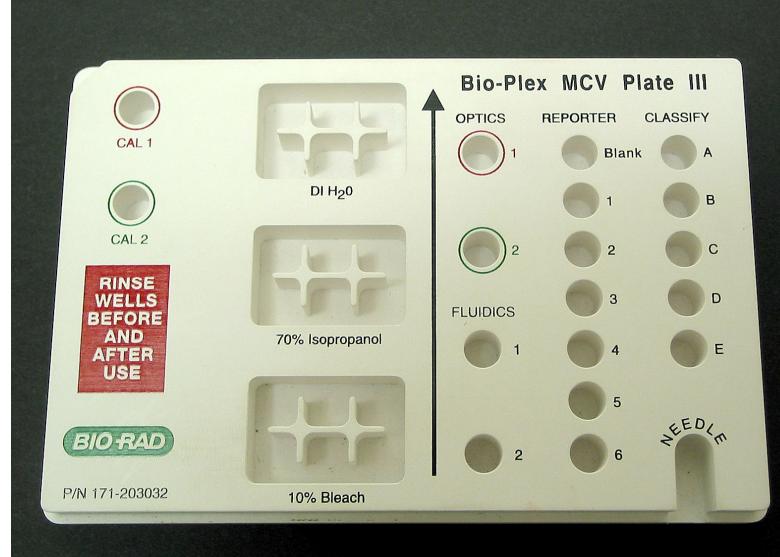
Bio-Plex Manager Software

Automated Start Up,





MCV Plate



MCV plate functions for maintenance, calibration, validation

- 1) Automated calibration and validation protocol,
- 2) System startup and shutdown routines,
- 3) Automated washes between assay plates
- 4) Easy adjustment of sample needle height.

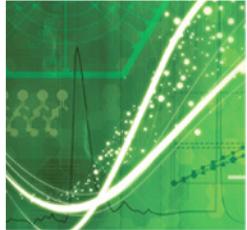


Validation Tools

- Verify that Bio-Plex system is performing according to specifications
- Used to troubleshoot instrument vs. assay problems
- Types of validation in Bio-Plex validation kit:
 - Optical validation (are lasers aligned)
 - Reporter Validation (linearity, sensitivity)
 - Classify validation (calls right beads right “name”)
 - Validation of fluidics integrity (bead carryover)



Validation Specifications			
Optics			
DD Median:	4774 - 6593	CL1 CV%:	3.00 - 7.00
CL1 Median:	3383 - 4135	CL2 CV%:	4.00 - 8.00
CL2 Median:	3520 - 4302	RP1 CV%:	5.00 - 10.00
RP1 Median:	15205 - 18583		
Fluidics			
Carryover:	< or = 3.5%		
Reporter			
Dynamic Range:	4.15 - 4.28	Accuracy:	> 90.00%
Sensitivity:	< 200 MESF	Linearity:	> 0.995
Slope:	0.0593 - 0.0799		
Classify			
Bead 34:	> 80.0%	DD Efficiency:	> or = 75.0%
Bead 38:	> 80.0%		
Bead 54:	> 80.0%		
Bead 73:	> 80.0%		
Bead 77:	> 80.0%		
Close			



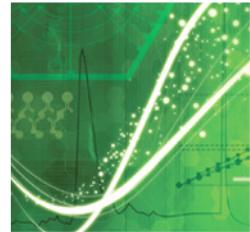
Bio-Plex Manager Software

Rapid and Simple Plate

The screenshot shows the Bio-Plex Manager Software interface. The main window title is "Protocol1 - Format Plate". On the left, there's a vertical list of steps: 1. Describe Protocol, 2. Select Analytes, 3. Format Plate, 4. Enter Standards Info, 5. Enter Controls Info, 6. Enter Sample Info, and 7. Run Protocol. Step 3 is highlighted with a blue border. Above this list are some icons and a dropdown menu set to "2". Below the steps is a "Plate Formatting" tab, which displays a 12x8 grid representing a plate layout. The columns are labeled 1 through 12, and the rows are labeled A through H. Each cell contains a number representing a sample ID or position. To the right of the main window is a "Quick Guide" window with a list of four items:

- 1. Start up
 Wash Between Plates
 Calibrate
- 2. New Protocol
 Open Protocol
- 3. Open Results
- 4. Shut Down
 Show at startup

The background of the software has a repeating "Bio-Plex" watermark.



Bio-Plex Manager Software

Easy to Use Reports

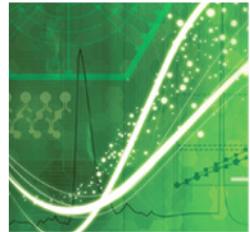
Bio-Plex Manager Desktop - [Human Cytokine 17-Plex Standard PMT (Read-Only) - Report Table]

File Edit View Table Options Tools Window Help

Results Raw Data Report Table Standard Curve Graphs

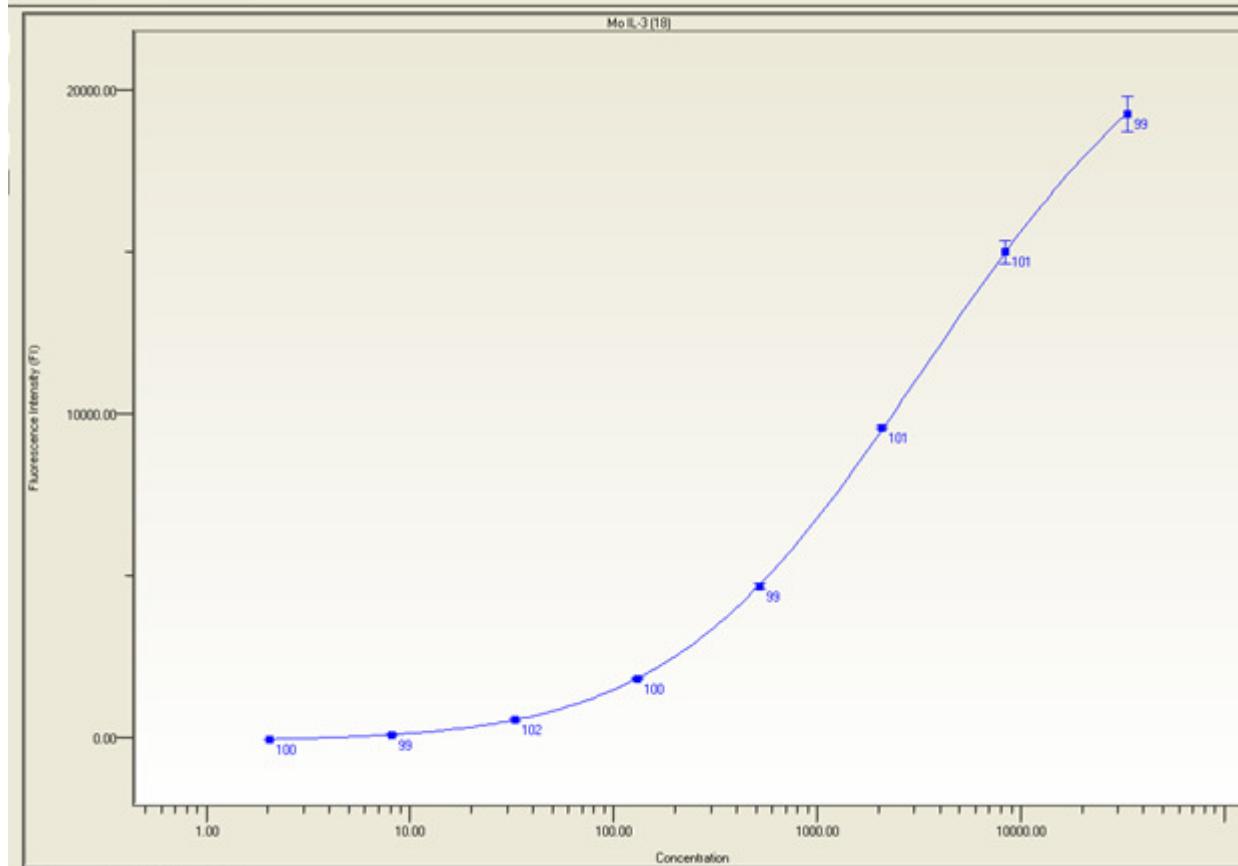
Analyte: Hu IL-10 (56)

	Type	Well	Description	Fl	Fl - Bkgd	Std Dev	%CV	Obs Conc	Exp Conc	(Obs/Exp) * 100	Conc in Range
1	B	A4,B4,C4		8.7	8.7	0.58	6.66				
2	S1	A5,B5,C5	Standard 1	12577.0	12568.3	428.27	3.39	31960.12	32000.00	100	31960.12
3	S2	D5,E5,F5	Standard 2	10048.2	10089.5	270.25	2.69	7889.98	8000.00	99	7889.98
4	S3	A6,G5,H5	Standard 3	6016.3	6007.7	39.69	0.66	2049.81	2000.00	102	2049.81
5	S4	B6,C6,D6	Standard 4	2308.3	2294.7	81.10	3.52	487.96	500.00	98	487.96
6	S5	B6,F6,G6	Standard 5	705.0	696.3	20.52	2.91	125.89	125.00	101	125.89
7	S6	A7,B7,H6	Standard 6	192.5	183.8	13.08	6.77	32.05	31.25	103	32.05
8	S7	C7,D7,E7	Standard 7	51.3	42.7	1.53	2.98	7.57	7.81	97	7.57
9	S8	F7,G7,H7	Standard 8	19.2	10.5	0.29	1.51	1.98	1.95	101	1.98
10	X1	D4,E4,F4	Sample 1	147.0	138.3	6.08	4.14	24.12			24.12
11	X2	A8,G4,H4	Sample 2	685.0	676.3	19.97	2.92	122.05			122.05
12	X3	B8,C8,D8	Sample 3	2609.0	2600.3	52.44	2.01	571.86			571.86



Bio-Plex Manager Software

Curve Fitting and Statistical Tools

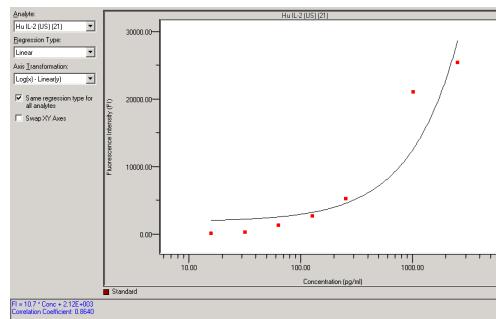


Brenden Scientific Weighted 5 Parameter Logistic Regression



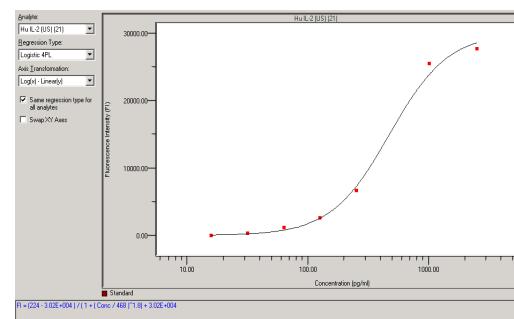
Superior standard curve

- Superior curve fitting
 - *Weighted 5PL algorithm from Brendan Scientific's StatLIA*



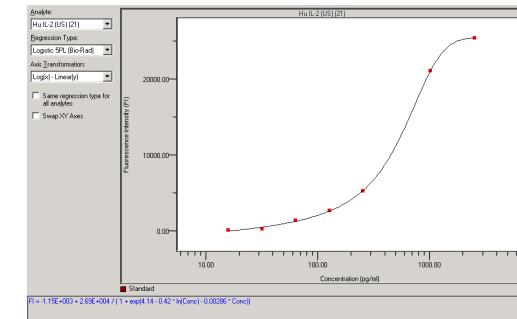
Linear

Obs Conc	Exp Conc	(Obs/Exp) * 100	Conc in Range	Dilution
				1.00
OOR	15.60	***	***	1.00
OOR	31.25	***	***	1.00
OOR	62.50	***	***	1.00
71.87	125.00	57	***	1.00
313.60	250.00	125	***	1.00
1782.87	1000.00	178	***	1.00
2189.46	2500.00	88	2189.46	1.00



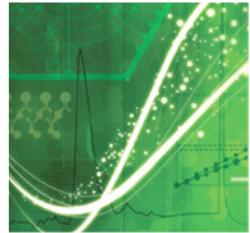
Logistic (4PL)

Obs Conc	Exp Conc	(Obs/Exp) * 100	Conc in Range	Dilution
				1.00
OOR	15.60	***	***	1.00
36.11	31.25	116	36.11	1.00
76.13	62.50	122	76.13	1.00
124.82	125.00	100	124.82	1.00
232.65	250.00	93	232.65	1.00
1217.16	1000.00	122	1217.16	1.00
1865.38	2500.00	75	1865.38	1.00



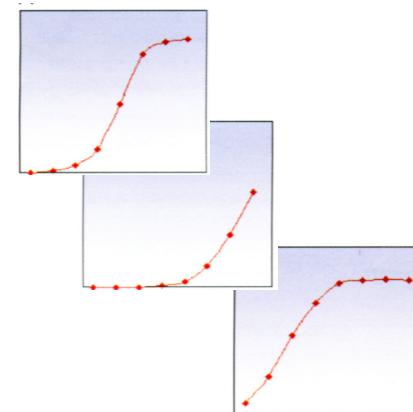
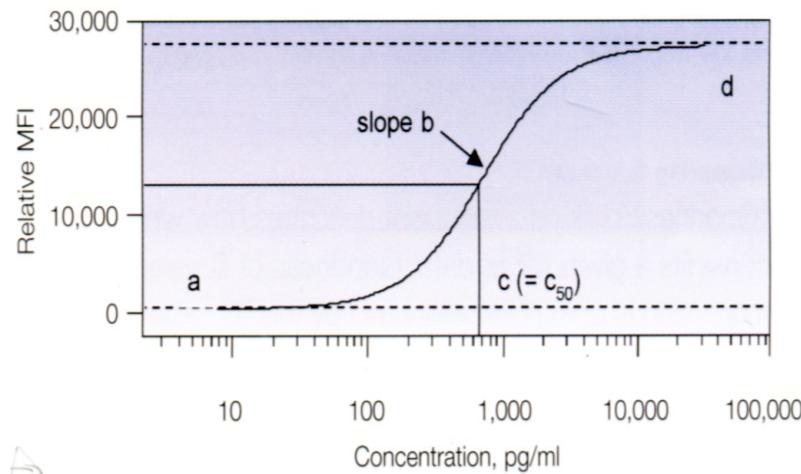
Logistic (5PL)

Obs Conc	Exp Conc	(Obs/Exp) * 100	Conc in Range	Dilution
				1.00
18.33	15.60	117	18.33	1.00
23.98	31.25	77	23.98	1.00
66.18	62.50	106	66.18	1.00
128.56	25.00	103	128.56	1.00
247.98	250.00	99	247.98	1.00
1000.53	1000.00	100	1000.53	1.00
2478.66	2500.00	99	2478.66	1.00



Superior standard curve

- Superior curve fitting
 - *Weighted 5PL algorithm from Brendan Scientific's StatLIA*



4PL

$$y = d + \frac{a - d}{\left[1 + \left(\frac{x}{c}\right)^b\right]}$$

Where:

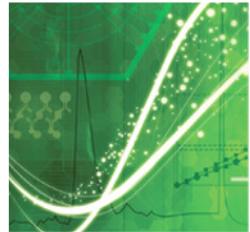
a = estimated response at zero concentration
b = slope factor
c = mid-range concentration (c_{50})
d = estimated response at infinite concentration

5PL

$$y = d + \frac{a - d}{\left[1 + \left(\frac{x}{c}\right)^b\right]^g}$$

Where:

a = estimated response at zero concentration
b = slope factor
c = mid-range concentration (c_{50})
d = estimated response at infinite concentration
g = asymmetry factor



Bio-Plex Manager Software

Easy to Use Reports

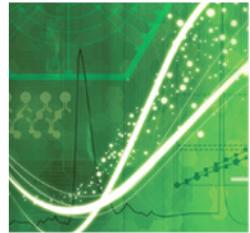
The screenshot shows the Bio-Plex Manager Desktop software interface. The title bar reads "Bio-Plex Manager Desktop - [Phosphoprotein 11-Plex (Read-Only) - Format Plate]". The menu bar includes File, Edit, View, Format Options, Tools, Window, and Help. The toolbar contains icons for file operations and analysis tools. The left sidebar under "Results" lists Raw Data, Report Table, Standard Curve, and Graphs. The main area displays a "Plate Formatting" grid for a 12x8 plate. The grid has columns labeled 1-12 and rows labeled A-H. Cells are color-coded: red for samples 1 through 8, and green for samples 9 through 16. A purple arrow points from the text "Samples" to the rightmost column of the grid.

	1	2	3	4	5	6	7	8	9	10	11	12
A			1	9								
B			2	10								
C			3	11								
D			4	12								
E			5	13								
F			6	14								
G			7	15								
H			8	16								

Reference 1

Reference 2

Samples



Bio-Plex Manager Software

Easy to Use Reports

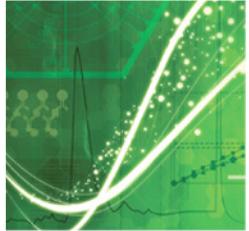
Bio-Plex Manager Desktop - [Phosphoprotein 11-Plex (Read-Only) - Report Table]

File Edit View Table Options Tools Window Help

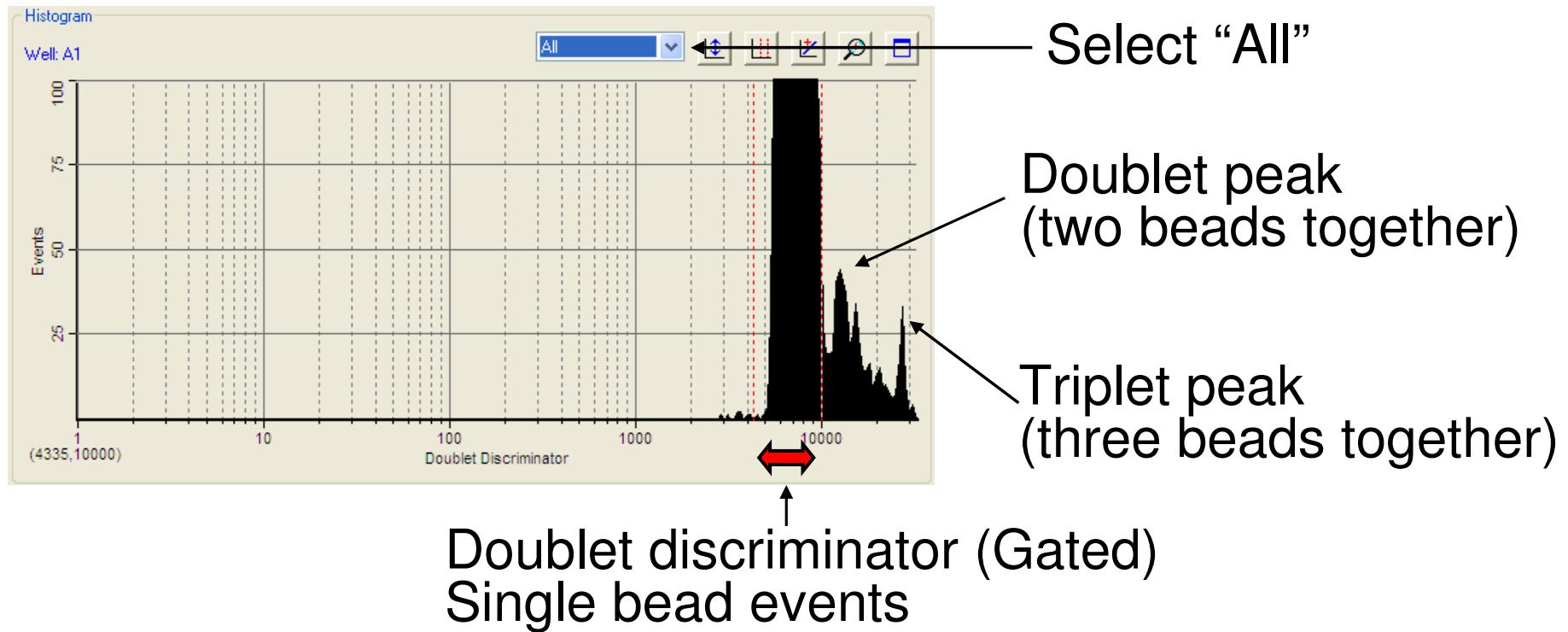
Raw Data Report Table Standard Curve Graphs

Analyte: p-ATF-2 (20)

Results	Type	Well	Description	Fl	Group	Ratio	Dilution
Raw Data	X1	A3	Blank	162.0	X1	1.00	1.00
Report Table	X2	B3	HeLa	1054.5	X1	6.51	1.00
Standard Curve	X3	C3	EGF/HEK	5758.5	X1	35.55	1.00
Graphs	X4	D3	UV/HEK	8843.0	X1	54.59	1.00
	X5	E3	TNF α /HeLa	6889.0	X1	42.52	1.00
	X6	F3	NGF β /PC12	7000.5	X1	43.21	1.00
	X7	G3	EGF/HeLa	2683.0	X1	16.56	1.00
	X8	H3	IL4/HeLa	1698.0	X1	10.48	1.00
	X9	A4	Blank	161.0	X9	1.00	1.00
	X10	B4	HeLa	1253.5	X9	7.79	1.00
	X11	C4	EGF/HEK	5709.0	X9	35.46	1.00
	X12	D4	UV/HEK	9259.0	X9	57.51	1.00
	X13	E4	TNF α /HeLa	7869.0	X9	48.88	1.00
	X14	F4	NGF β /PC12	7380.0	X9	45.84	1.00
	X15	G4	EGF/HeLa	2658.5	X9	16.51	1.00
	X16	H4	IL4/HeLa	1685.5	X9	10.47	1.00



Doublet discriminator (DD) Gated





Internal / External Control

Data Normalization using internal controls (housekeeping genes) or external/exogenous controls.

The image displays two windows from the Bio-Plex Manager software:

Normalization Settings window:

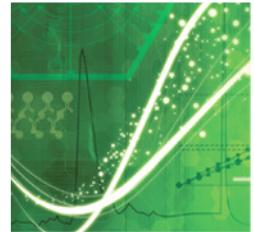
- Internal Control/Housekeeping Gene(s):
 - None
 - Single (selected): Mo IL-1a
 - Multiple (selected): Mo IL-1a, Mo IL-1b
- Control Sample:
 - None
 - Single
- Instructions:
 - The Internal Control/Housekeeping Gene field accounts for differences in the amount of sample material.
 - The Control Sample field allows you to express all samples as a ratio to the assigned control.
 - For more details, click Help.

Select Internal/Housekeeping Gene Controls window:

Available:	Selected:
Region Analyte	Region Analyte
53 Mo IL-1a	
19 Mo IL-1b	
36 Mo IL-2	
18 Mo IL-3	
32 Mo IL-4	
52 Mo IL-5	
38 Mo IL-6	
33 Mo IL-9	
56 Mo IL-10	
76 Mo IL-12(p40)	
58 Mo IL-12(p70)	

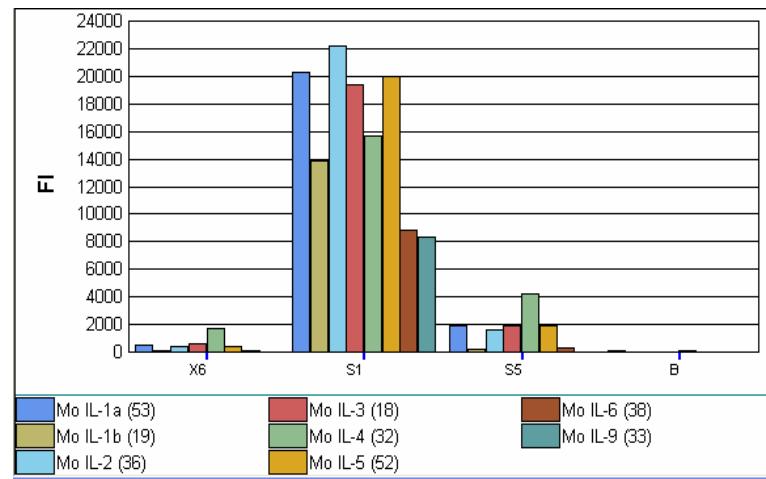
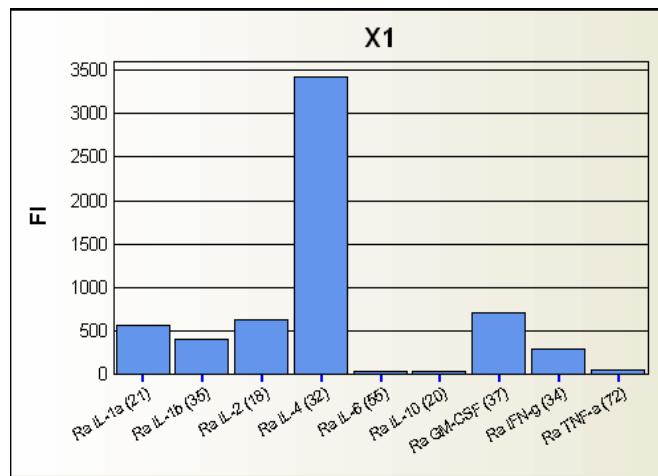
Buttons: OK, Cancel, Add >>, << Remove, << Remove All.

Benefit: This features allows users to establish a comparison parameter. Thus dramatically increasing confidence in the obtained results

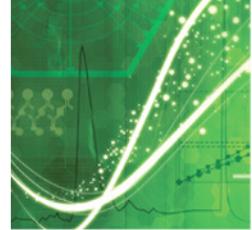


Graphing Tool

Graphing capability, for visually comparing sample results between samples and/or across analytes.



Benefit: This is a great data visualization capability, it allows users to quickly make sense of the data using a customizable graph.



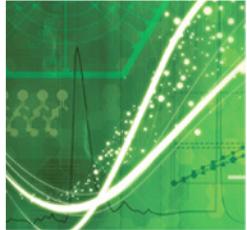
Bio-Plex Manager Software

- Reporting Options - Export to Excel / text file.

A	B	C	D	E	F	G	H	I	J
1	File Name: D:\BioPlex\xMAP Bake-off 2004\R&D.rbx								
2	Acquisition Date: 01-Sep-2004, 03:42 PM								
3	Reader Serial Number: LX10003219008								
4	Plate ID:								
5	RP1 PMT (Volts): 601.22								
6	RP1 Target: 3885								
7									
8									
9	Type Well	Description	hu IL-1 beta (6)	hu IL-2 (17)	hu IL-4 (21)	hu IL-5 (9)	hu IL-6 (32)	hu IL-8 (36)	hu TNF-alpha (77)
10	B A3,A4,A5,E6,E7,E8,H6,H7,H8		FI	FI	FI	FI	FI	FI	FI
11	S1 A1,A2	R&D stds	3431.5	5714.5	2724	9972.5	5770	8413	7046
12	S2 B1,B2	R&D stds	2189.3	2209.5	962.8	4789.3	2907	5469.5	3358.5
13	S3 C1,C2	R&D stds	980	716	271.5	1800.8	1343.3	2800	1238.8
14	S4 D1,D2	R&D stds	403.5	199.5	66.8	549.5	505.3	1265.5	435.5
15	S5 E1,E2	R&D stds	183.3	66	23.3	140.5	176.5	508	155
16	S6 F1,F2	R&D stds	96	23	10.5	36.5	61	194	69.8
17	S7 G1,G2	R&D stds	69.3	10.5	7.5	11	23	72.3	41.5
18	C1 F9,F10	R&D stds	64	10	7	10.5	23	73.3	42
19	C2 G9,G10	R&D stds	94	22	10.3	33.3	61.8	188.3	64.5
20	C3 H9,H10	R&D stds	171.8	58	20	132.3	171	508.5	152
21	C4 A9,A10	R&D stds	394	192.5	63.5	540	493.8	1214.5	410
22	C5 B9,B10	R&D stds	937.5	656.3	272	1897.5	1293	2873.5	1210.5
23	C6 C9,C10	R&D stds	1056.8	1128.5	513.8	2266.3	1451	2721	1737.5
24	C7 D9,D10	R&D stds	4190.5	5725.8	2782.3	9389	5538.8	8277	7106.3
25	C8 B11,B12	R&D stds	62.5	10.3	8.5	10	24	70	40
26	C9 C11,C12	R&D stds	85	21.5	9	32.3	61.8	189.3	68.8
27	C10 D11,D12	R&D stds	177.5	61	21	138	184.5	524.5	153.8
28	C11 E11,E12	R&D stds	401.5	186.8	65	543.8	518	1225.5	425.8
29	C12 F11,F12	R&D stds	957	634.8	274.8	1855	1277.3	2635	1212.5
30	C13 G11,G12	R&D stds	2075	2228.5	1010	5056	2936.5	5056	3347.5
31	C14 H11,H12	R&D stds	4182	5762	2744	10105.5	5357.5	8371	6924.5
32	C15 B3,B4,B5	Bio-Rad stds in serum	9772.2	6304.5	2331	12370.7	1183.7	8932.5	4518.2
33	C16 C3,C4,C5	Bio-Rad stds in serum	2269.5	418.2	111	1918	81.3	2422.5	381
34	C17 D3,D4,D5	Bio-Rad stds in serum	716.3	70.3	17.8	322.7	27.5	690.5	118.8
35	C18 E3,E4,E5	Bio-Rad stds in serum	91.7	6.7	6	10.2	6.3	55.5	31.7

Why Choose Bio-Rad ?





Bio-Plex Nucleic Acid Products



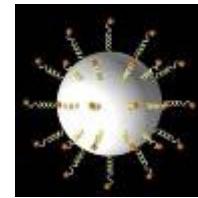
Amplification
Reagents



Extraction
Reagents



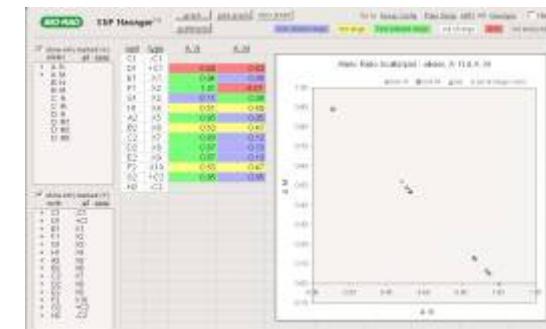
Thermal
Cyclers



Beads and
Coupling Protocols

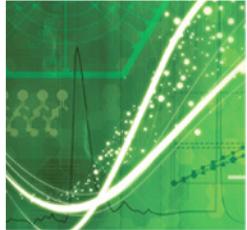


Bio-Plex System and
Support



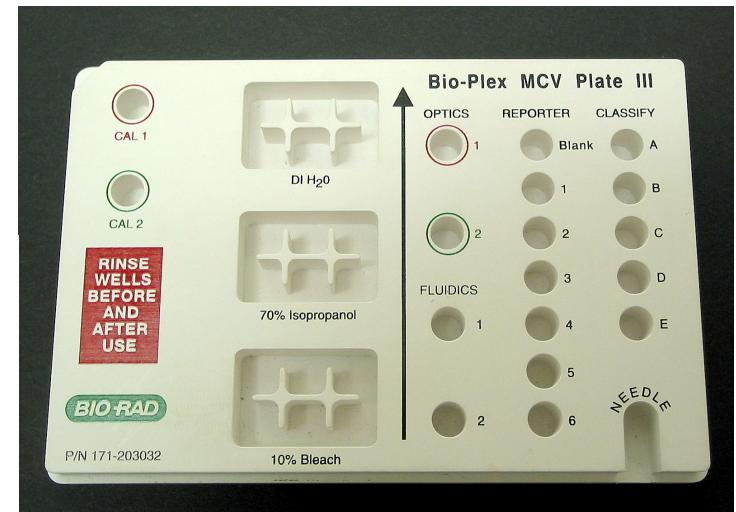
SNP Manager
Software

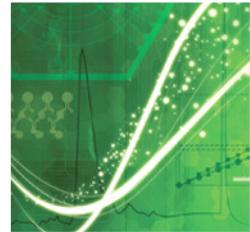
Unique



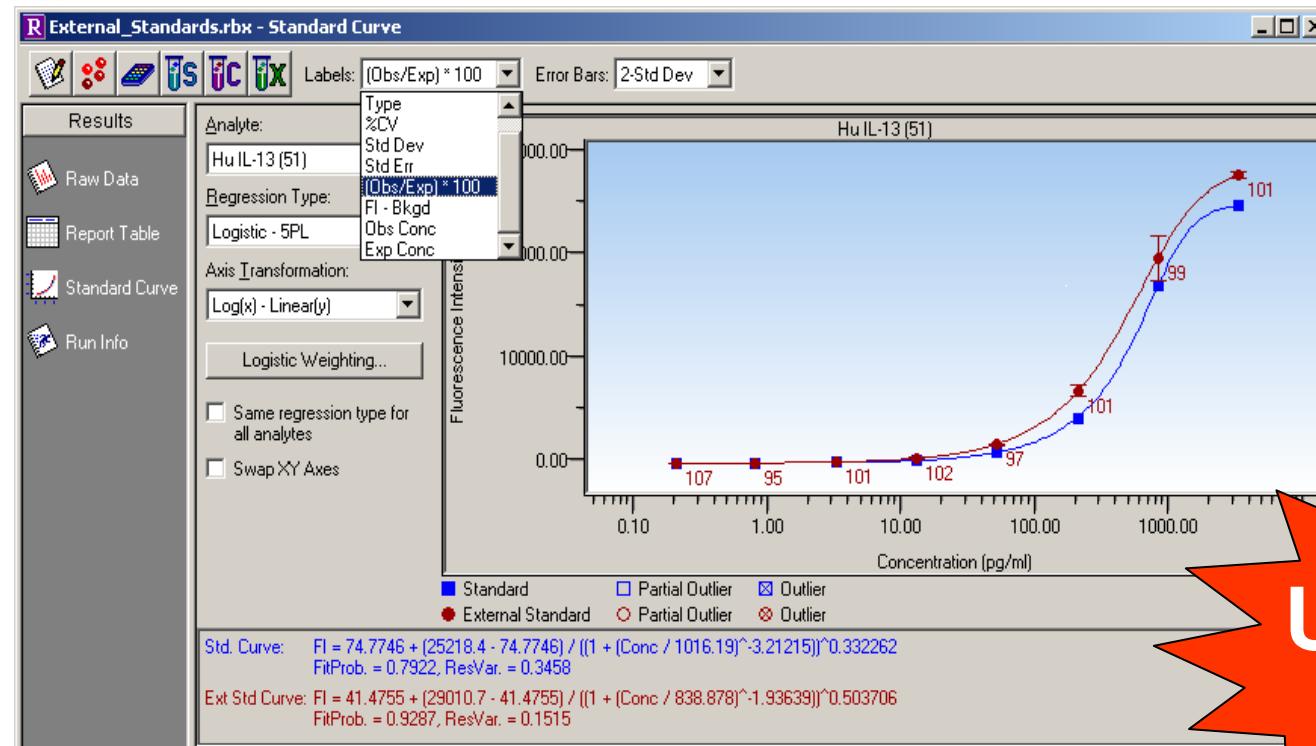
Validation & Calibration Kits

Unique

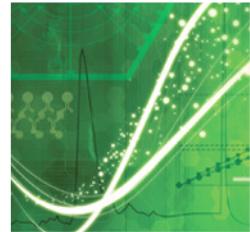




Unique Analysis Tools



- Brendan Scientific StatLIA 4PL and 5 PL weighted curve-fitting model
- Reanalyze result for modification of DD gates



Automatic Workflow

- Two Wash Station Options
 - Bio-Plex Pro Wash Station
 - Magnetic carrier only
 - For Bio-Plex Pro assays
 - Bio-Plex Pro II Wash Station
 - Magnetic and Vacuum carrier
 - For all xMAP assays
- No Programming Required

BIO-RAD

Bio-Plex® Pro
Wash Station

BIO-RAD

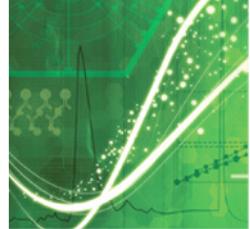
Bio-Plex® Pro II
Wash Station



Unique

Bio-Plex Pro Wash Station Selection Guide

	Bio-Plex Pro Wash Station	Bio-Plex Pro II Wash Station
Bio-Plex assays (polystyrene beads)	—	•
Polystyrene xMAP microspheres	—	•
Bio-Plex Pro assays (magnetic beads)	•	•
Bio-Plex® Precision Pro™ assays (magnetic beads)	•	•
MagPlex microspheres (magnetic beads)	•	•



Suspension Bead Array

Technology Benefits of xMAP Technology

Economics

- Labor, reagents, sample

Flexibility

- Many assays commercially available
- User can create custom assays

Accuracy and reproducibility

- CVs are under 10%
- Correlation with ELISA, Western Blot
- Multiplexing allows internally controlled experiments

A world of information...

Assays Tools Technology Software Support

Literature, Manuals, and Glossary

Events

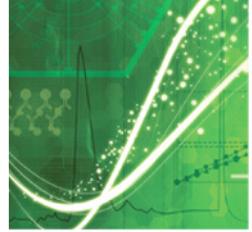
View Archive of Recent Live Webcast. Case Studies in Multiplex Protein Analysis

Bio-Plex Phosphoprotein Detection

x-Plex Assays

New Literature (PDF)

Cytokine Assays



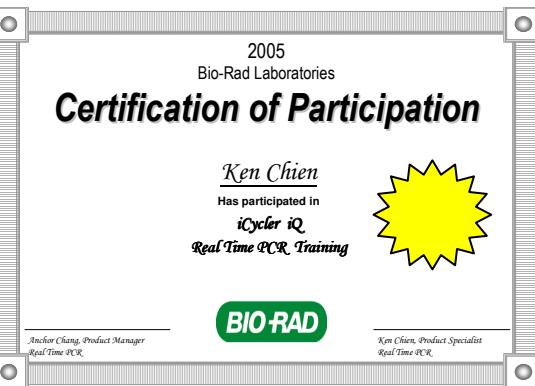
堅強的團隊: Capital Sales Team SEA, Taiwan

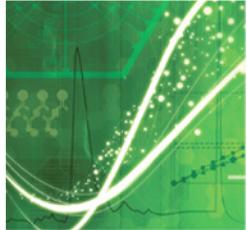
- Sales:
 - 2 , Master degree, Level 1 trouble shooting
- FAS:
 - 4, Master degree, Training , advance trouble shooting
- FSE:
 - 4, > 5 years certified engineers

Unique



訓練剪影





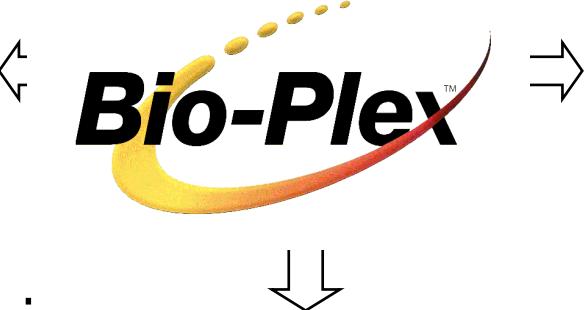
The Bio-Plex Solution



Software
✓ Instrument
Control
✓ Data Analysis



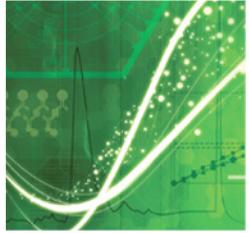
Hardware



Beads & Assays
✓ Cytokines
✓ Phosphoproteins
✓ Amine Coupling Kit
✓ Calibration/Validation

Service & Support

www.bio-rad.com/bioplex



Questions?