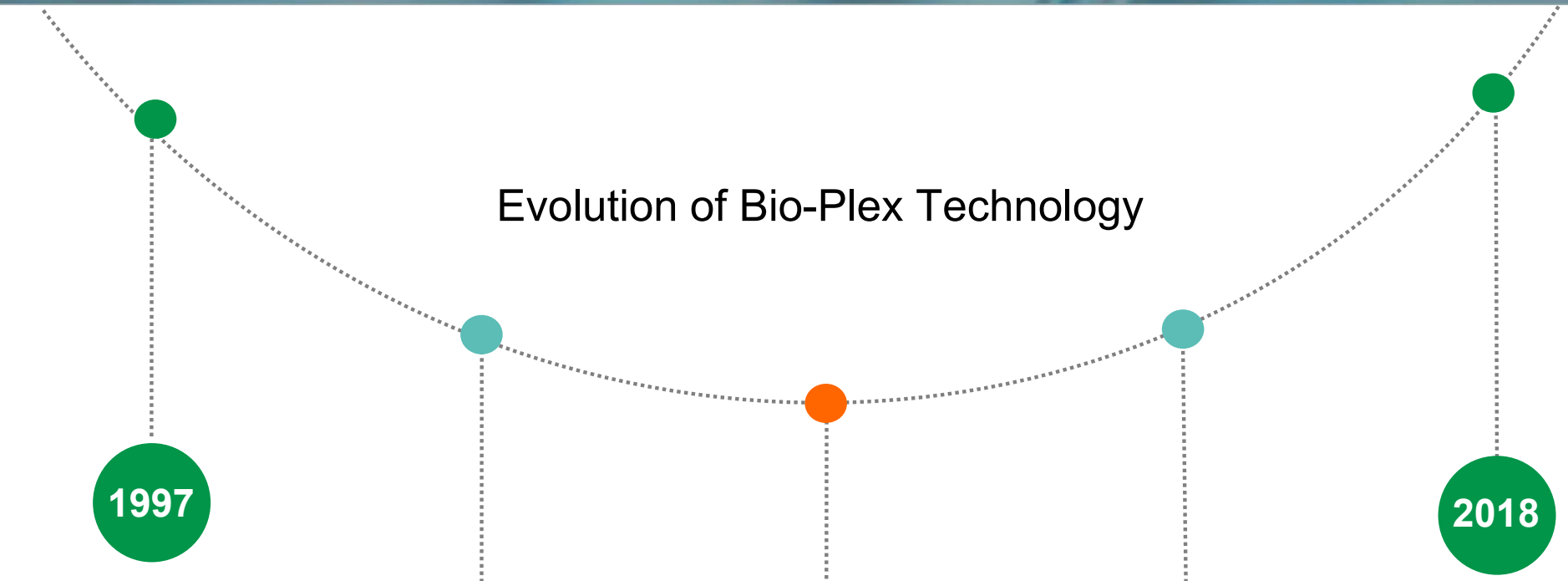
Bio-Plex200 System overview

Kamol Rodyou
Field and Application Specialist

> 20 Years of Multiplex Assay Expertise



Early Beginnings
Bio-Rad Licensed xMAP Technology

Launched Bio-Plex 200

Expanding Assay Offerings
Bio-Plex Pro TGF Beta
Bio-Plex Rat Cytokines
Bio-Plex Data Pro Software
Bio-Plex Manager 6.1

Growing User Base
Over 3,000 Bio-Plex 200 Systems placed world wide
Over 17,000 scientific publications

Continued Evolution
Bio-Plex Manager 6.2
Hu Cytokine Screening Panel

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Bio-Plex Core Technology

- xMAP® Multiplex suspension array technology - Luminex
- Microspheres or bead-based immunoassay – coupled with either DNA or protein capture reagent (antibody)
- Analytes are captured by specific biomolecular reaction on magnetic bead surface such as antigen-antibody interaction
- Assay results are quantified using a fluorescent reporter tag
- Simultaneous analysis of up to 100-500 different analytes in 96-well format

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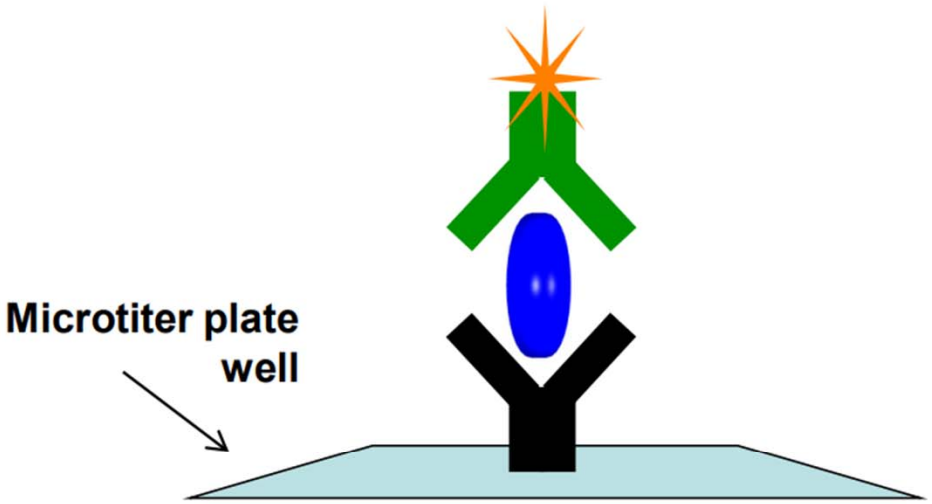
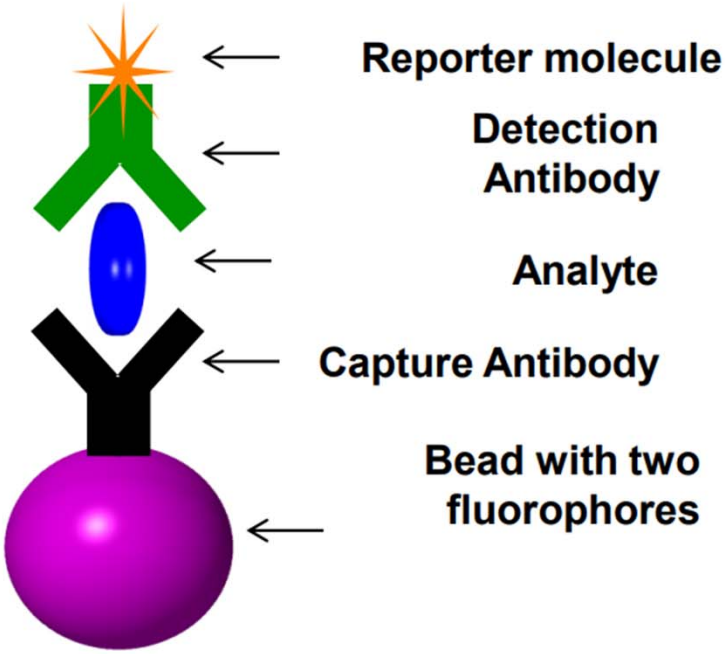


ELISA



ELISA on a bead

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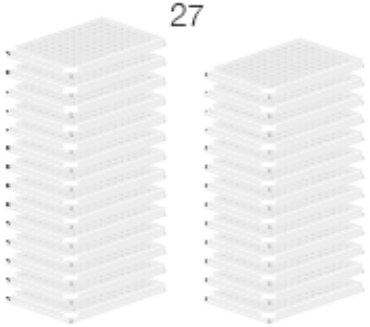
Multiplexing Increases Throughput

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

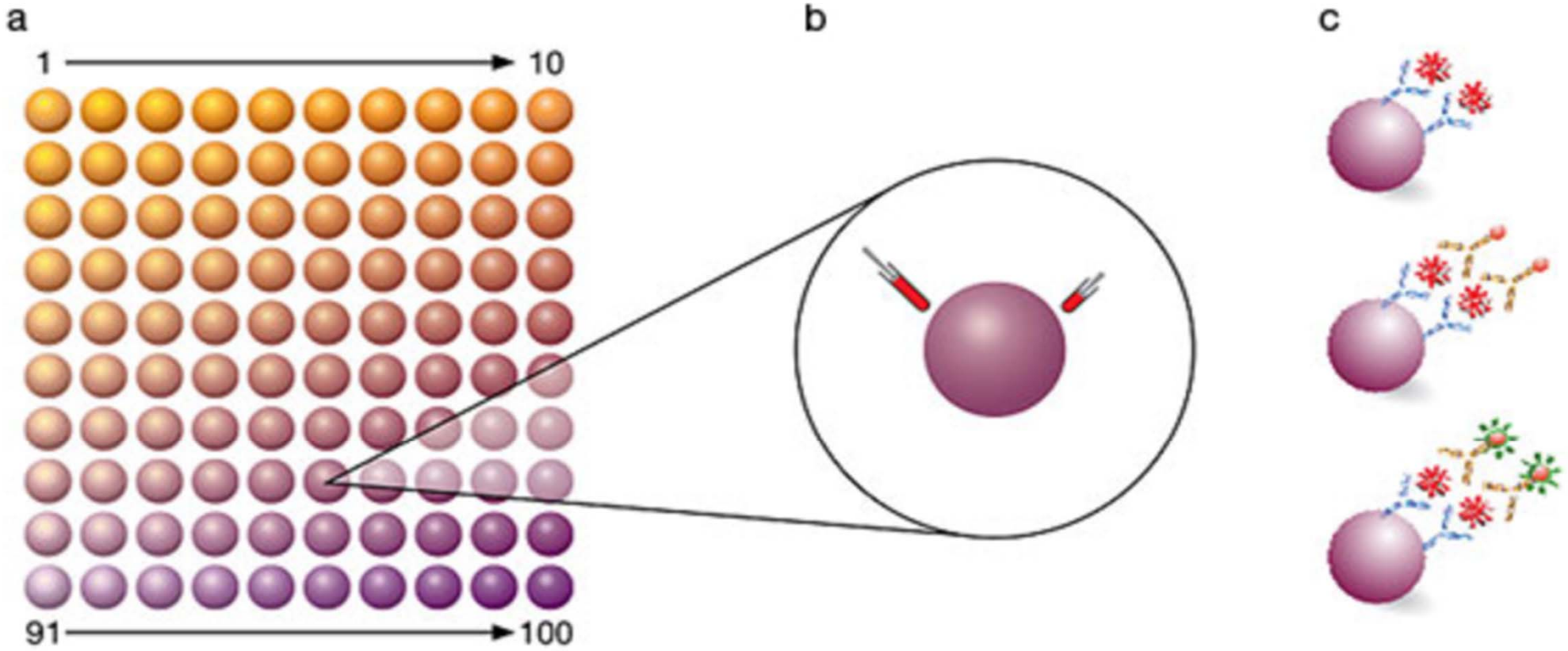
The Power of the Bio-Plex System

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.

What is xMAP Luminex Technology?



- Beads are injected with 2 dyes, the ratio identifies the bead creating a bead map of up to 100 discernable beads
- The reporter molecule SAPE intensity gives intensity that can be translated into concentration

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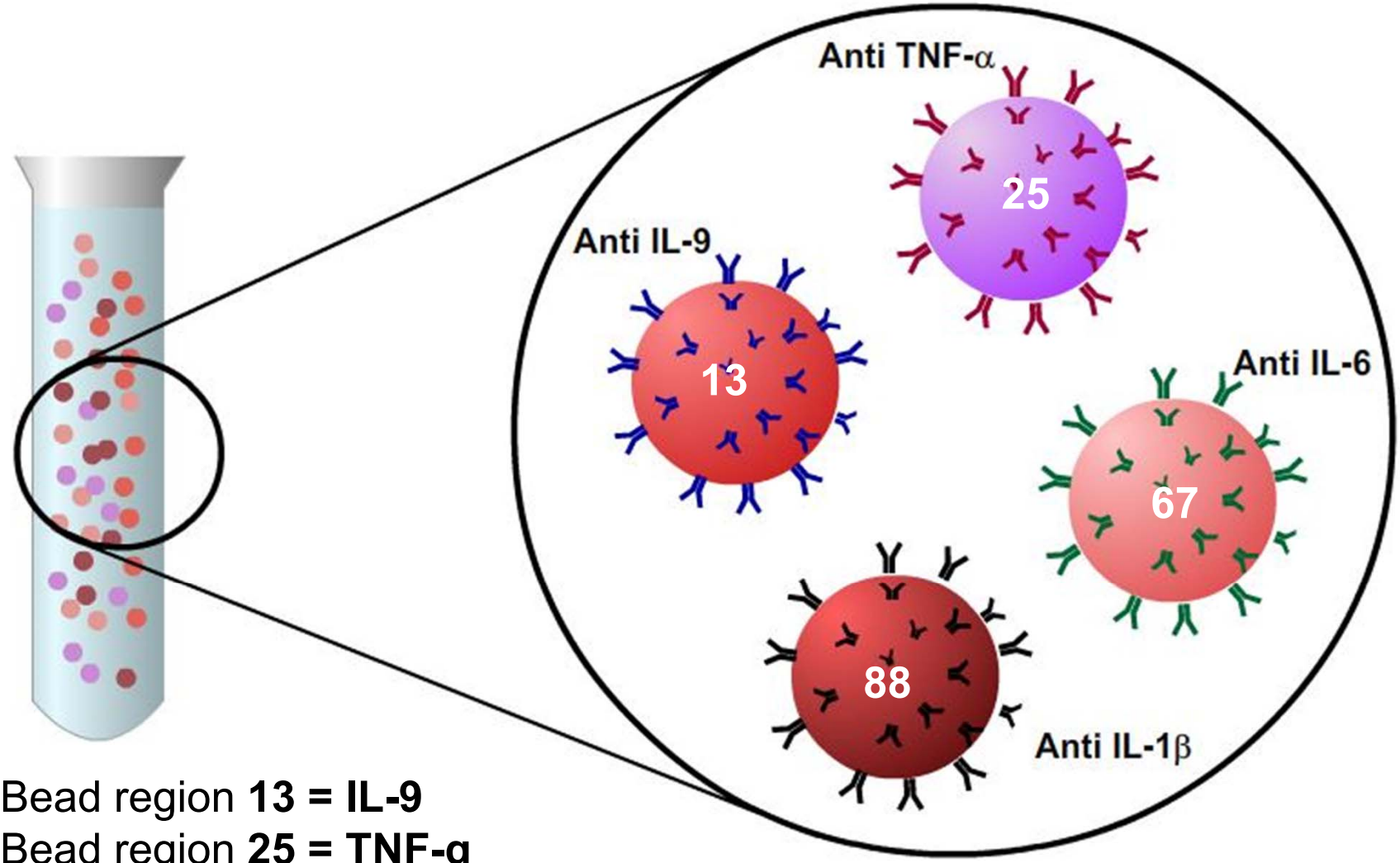
What is xMAP Luminex Technology?



Bead region **13 = IL-9**
Bead region **25 = TNF-α**
Bead region **67 = IL-6**
Bead region **88 = IL-1β**

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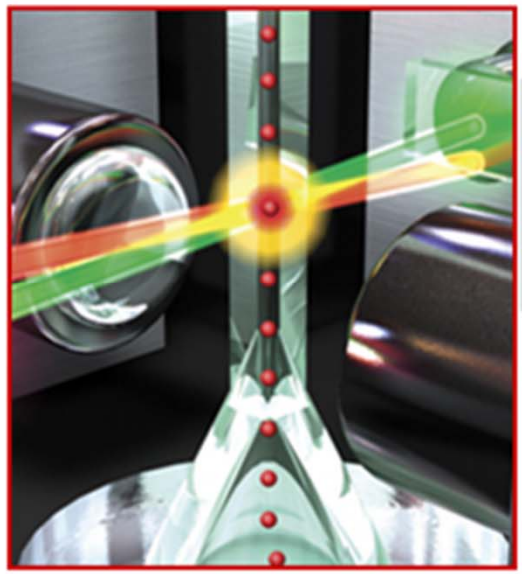
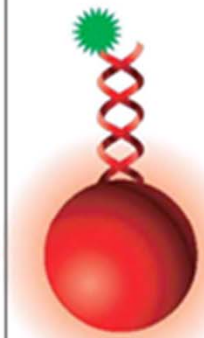
What is xMAP Luminex Technology?

- Fluorescent signal reading system



Interrogate label with green laser (525 nm)

Interrogate bead with red laser (635 nm)



Quantify binding events (RP1)

Identify bead region based on internal dye concentrations (CL1 & CL2)

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Bio-Plex system

Bio-Plex Instruments

Bio-Plex Readers and Tools include the Bio-Plex 200 and Bio-Plex 3D Systems, Bio-Plex Pro Wash Station, and maintenance, calibration, verification, and validation kits.

Bio-Plex Software

Bio-Plex Manager and Bio-Plex Data Pro Software make it simple to run instruments using xMAP technology and analyze multiplex data.

Bio-Plex Standard Lot Data

Download data for released standard lots for import into Bio-Plex Manager Software.

Bio-Plex Assays, Kits, and Reagents

Bio-Plex Multiplex immunoassays use Luminex magnetic beads for the quantification of over 450 biologically relevant targets: assays for inflammation, disease, cancer, cell signaling and growth, apoptosis, toxicity, and more. Choose assays in premade and custom configurations, or develop your own assays for new targets.

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Bio-Plex Assay configuration options

- **OTS (Off the shelf)**
  - All-in-one kits contain the largest number of analytes

Express Assay

- **Express** – "You Mix"
  - Configure custom analytes online, received individual vials of beads and detection plus reagent kit

Pre-Blended

- **x-Plex –** "We Mix"
  - Custom blend of analytes is built by Bio-Rad and undergoes lot-specific validation prior to shipment.

- **A la carte** – Individual components can be ordered to allow flexibility in day to day assay runs.

www.bio-rad.com/bio-plex

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Bio-Plex Pro human cytokine assays: Fixed panels

Human Group I	8-Plex Panel (M50-000007A)	17-Plex Panel (M50-00031YV)	27-Plex Panel (M50-0KCAF0Y)	Th1/Th2 Panel (M50-00005L3)	Singleplex/ x-Plex™ Bead Regions*
IL-1β		●	●		● 39
IL-1ra			●		● 25
IL-2	●	●	●	●	● 38
IL-4	●	●	●	●	● 52
IL-5		●	●	●	● 33
IL-6	●	●	●		● 19
IL-7		●	●		● 74
IL-8	●	●	●		● 54
IL-9			●		● 77
IL-10	●	●	●	●	● 56
IL-12 (p70)		●	●	●	● 75
IL-13		●	●	●	● 51
IL-15			●		● 73
IL-17		●	●		● 76
Basic FGF			●		● 44
Eotaxin			●		● 43
G-CSF		●	●		● 57
GM-CSF	●	●	●	●	● 34
IFN-γ	●	●	●	●	● 21
IP-10			●		● 48
MCP-1 (MCAF)		●	●		● 53
MIP-1α			●		● 55
MIP-1β		●	●		● 18
PDGF-BB			●		● 47
RANTES			●		● 37
TNF-α	●	●	●	●	● 36
VEGF			●		● 45

Human Group II	21-Plex Panel plus ICAM-1 and VCAM-1 (MF0-005KMII)	Singleplex/ x-Plex Bead Regions*
IL-1α	●	● 63
IL-2Rα	●	● 13
IL-3	●	● 64
IL-12 (p40)	●	● 28
IL-16	●	● 27
IL-18**	●	● 42
CTACK	●	● 72
GRO-α	●	● 61
HGF	●	● 62
ICAM-1***		● 12
IFN-α2	●	● 20
LIF	●	● 29
MCP-3	●	● 26
M-CSF	●	● 67
MIF	●	● 35
MIG	●	● 14
β-NGF	●	● 46
SCF	●	● 65
SCGF-β	●	● 78
SDF-1α	●	● 22
TNF-β	●	● 30
TRAIL	●	● 66
VCAM-1***		● 15

Bio-Plex Pro™ Human Cytokine Screening Panel

Expand your biomarker screening capabilities, shorten your time to discovery, and gain confidence in your data with the Bio-Plex Pro Human Cytokine Screening Panel 48-Plex.

Panel Contents			
CTACK	IL-2	IL-13	MIP-1α
Eotaxin	IL-2Rα	IL-15	MIP-1β
FGF basic	IL-3	IL-16	β-NGF
G-CSF	IL-4	IL-17	PDGF-bb
GM-CSF	IL-5	IL-18	RANTES
GRO-α	IL-6	IP-10	SCF
HGF	IL-7	LIF	SCGF-β
IFN-α2	IL-8	MCP-1	SDF-1α
IFN-γ	IL-9	MCP-3	TNF-α
IL-1α	IL-10	M-CSF	TNF-β
IL-1β	IL-12(p70)	MIF	TRAIL
IL-1ra	IL-12p40	MIG	VEGF

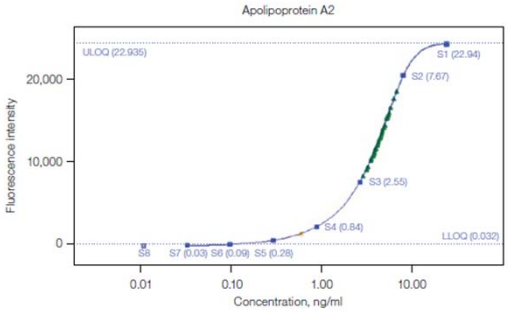
Assay Performance

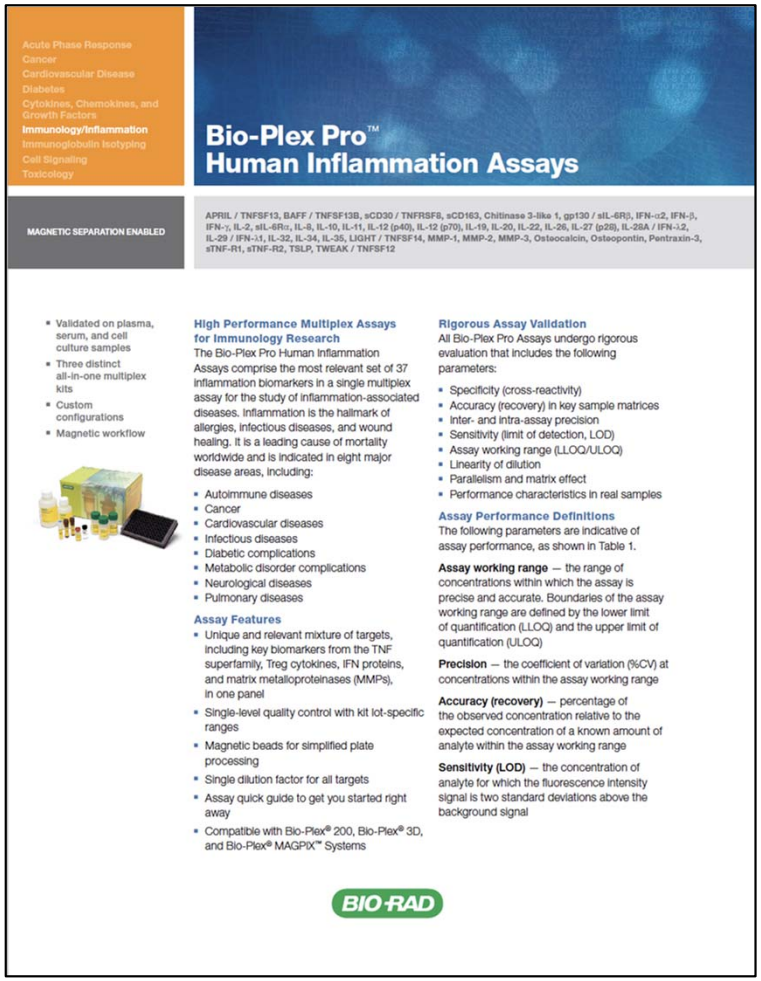
Assay performance characteristics:

1. Accuracy
2. Assay Working Range
3. Sensitivity
4. Reproducibility
5. Linearity of dilution

Table 1. Representative performance characteristics.

Analyte	Assay Working Range, pg/ml* LLOQ	ULOQ	Assay Sensitivity, pg/ml* LOD	Assay Precision* Intra-Assay %CV	Inter-Assay %CV	37 1
APRIL / TNFSF13	3,440.1	2,507,800.0	190.0	4.2	17.3	
BAFF / TNFSF13B	91.5	200,008.9	34.7	1.6	5.2	
sCD30 / TNFRSF8	53.4	12,973.1	1.0	2.5	5.0	
sCD163	1,338.7	975,916.6	16.8	4.2	8.1	
Chitinase 3-like 1	36.7	80,275.3	10.3	2.5	6.0	
gp130 / sIL-6Rβ	257.6	187,785.6	16.9	2.3	5.9	
IFN-α2	3.1	6,702.2	0.7	3.6	10.0	
IFN-β	0.9	1,872.8	2.0	3.0	11.2	
IFN-γ	6.3	13,694.9	0.05	3.7	7.3	
IL-2	1.2	2,662.7	0.1	3.3	10.3	
sIL-6Rα	18.6	40,675.1	1.5	3.1	3.2	
IL-8	4.5	9,762.7	2.7	4.5	7.7	
IL-10	1.7	3,781.2	0.6	3.0	8.5	
IL-11	0.1	207.1	0.05	3.6	16.9	
IL-12 (p40)	5.5	12,039.9	5.6	3.5	6.3	
IL-12 (p70)	1.3	908.0	0.1	3.5	5.5	
IL-19	8.5	6,223.8	0.2	3.8	8.4	
IL-20	2.7	5,809.9	3.6	2.3	11.1	
IL-22	5.3	11,511.0	1.1	3.2	6.5	
IL-26	8.2	5,969.7	1.2	3.1	10.9	
IL-27 (p28)	3.8	8,397.0	0.1	3.0	6.5	
IL-28A / IFN-λ2	10.7	7,813.5	1.8	4.3	7.9	
IL-29 / IFN-λ1	5.2	11,431.4	1.6	4.4	3.9	
IL-32	3.9	8,586.5	12.3	2.5	8.8	
IL-34	61.9	45,142.7	51.9	3.2	6.7	
IL-35	20.6	45,037.6	3.7	2.6	9.1	
LIGHT / TNFSF14	14.8	3,585.3	10.2	3.1	10.4	
MMP-1	106.8	233,460.8	33.7	4.8	9.0	





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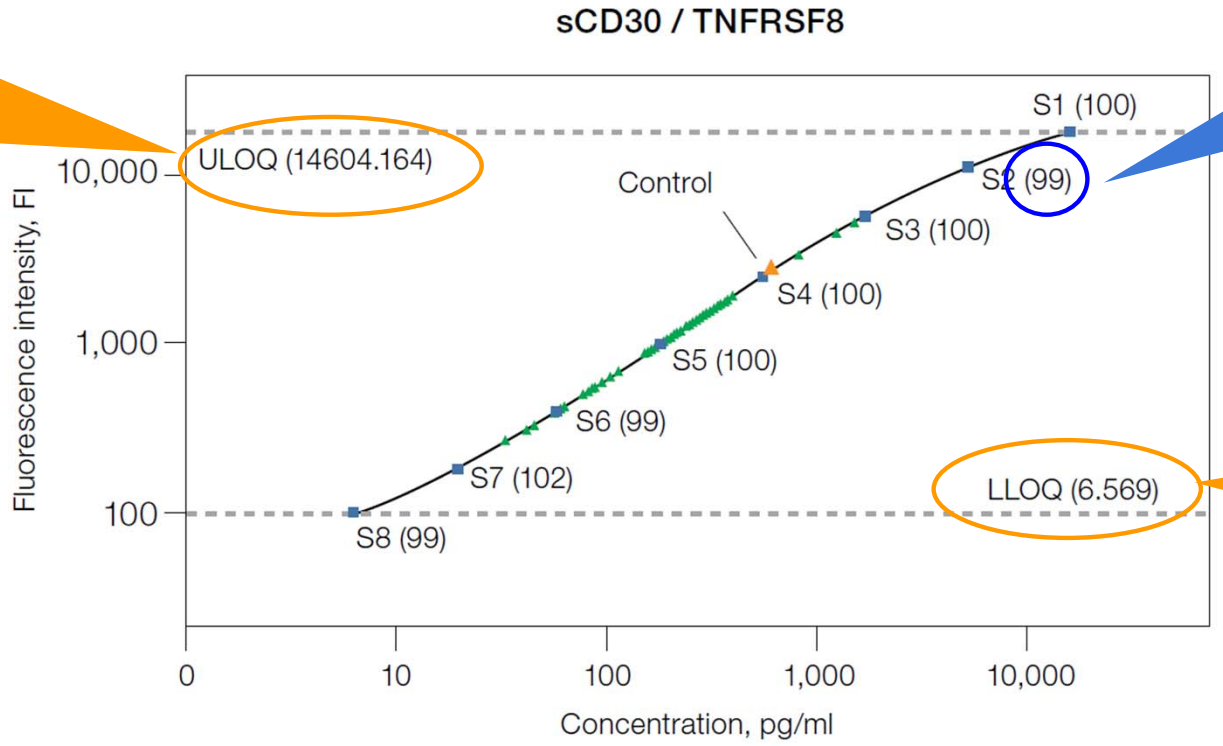
Assay Performance

Assay performance characteristics:

1. **Accuracy**
2. Assay Working Range
3. Sensitivity
4. Reproducibility
5. Linearity of Dilution

Upper
Limit
Of
Quantitation



%
Recovery

Lower
Limit
Of
Quantitation

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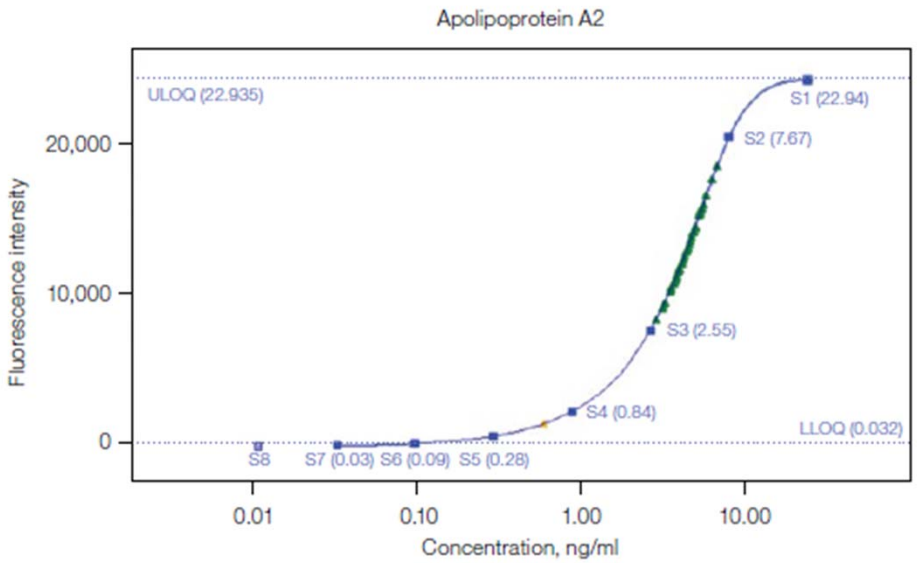
Assay Performance

Assay performance characteristics:

1. Accuracy
2. **Assay Working Range**
3. **Sensitivity**
4. Reproducibility
5. Linearity of dilution



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Assay Performance

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3. Sensitivity
4. **Reproducibility**
5. Linearity of dilution

Analyte	Alternative Names	Bead Region	Assay Working Ranges, ng/ml		Assay Sensitivity, ng/ml	Assay Precision	
			LLOQ*	ULOQ*	LOD**	Intra-Assay %CV	Inter-Assay %CV
Apolipoprotein A1	Apo A1	22	0.059	70	0.045	4	7
Apolipoprotein A2	Apo A2	26	0.032	36	0.016	6	15
Apolipoprotein B	Apo B	44	0.41	360	0.22	6	12
Apolipoprotein C1	Apo C1	36	0.030	17	0.0082	3	5
Apolipoprotein C3	Apo C3	39	0.023	28	0.013	3	10
Apolipoprotein D	Apo D	12	0.055	30	0.027	3	9
Apolipoprotein E	Apo E	38	0.021	12	0.012	4	6
Apolipoprotein H	Apo H	75	0.15	210	0.11	3	8
Apolipoprotein J	Clusterin/Apo J	48	0.12	170	0.078	2	8
C-reactive protein	CRP	78	0.019	11	0.0087	3	5

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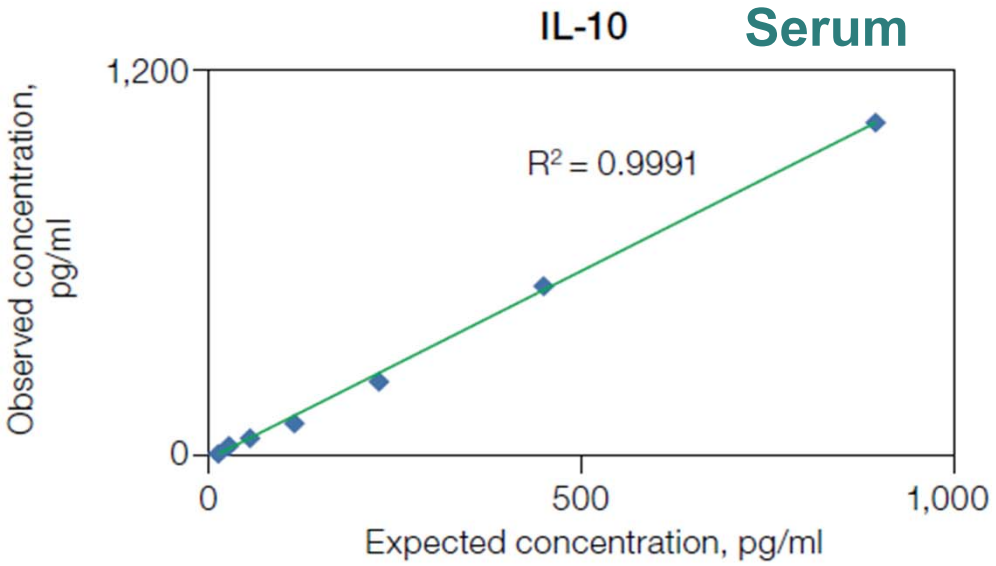
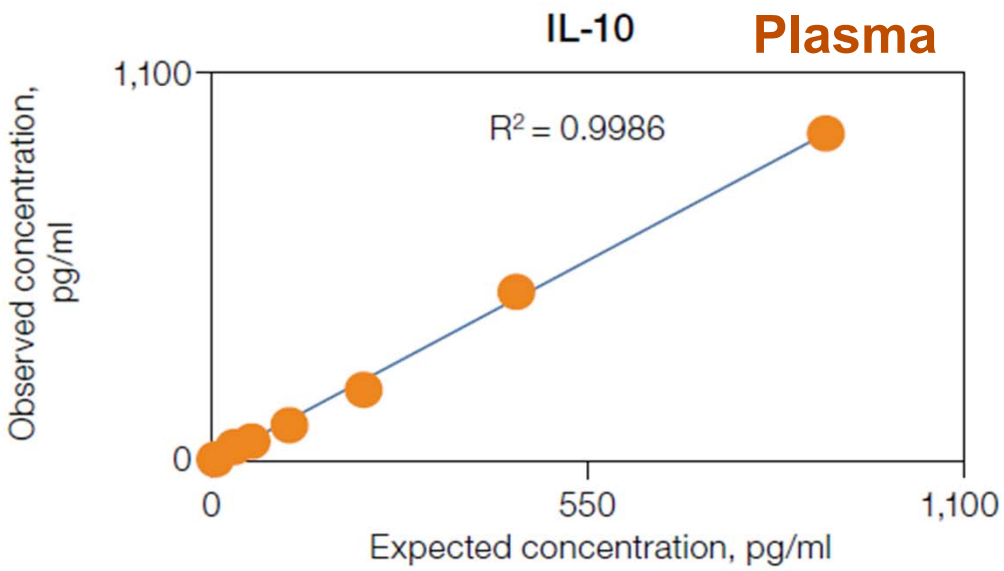
5. **Linearity of dilution**





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Bio-Plex Assay Overview

- Instrument preparation
  - instrument warm up
  - instrument calibration
  - instrument validation
- Assay preparation
  - Standard and reagent
  - Sample preparation
  - Bead preparation
- Assay workflow
- Reading signal by Bio-Plex200
  - Protocol setup
- Result interpretation by Bio-Plex Manager 6.2

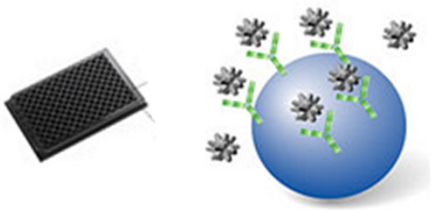
Summary of Bio-Plex Assay Workflow

Add 50 µl 1x beads to wells

Wash 2 x 100 µl

Add 50 µl standards, samples, controls, incubate 1 hr at RT with shaking at 850 rpm

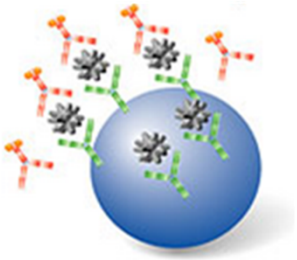
Mix antibody coated beads with samples, standards & controls



Wash 3 x 100 µl

Add 25 µl 1x detection antibody, incubate 30 min at RT with shaking at 850 rpm

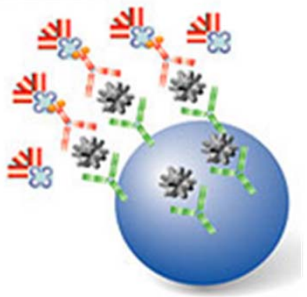
Add specific biotinylated detection antibodies



Wash 3 x 100 µl

Add 50 µl 1x streptavidin-PE, incubate 10 min at RT with shaking at 850 rpm

Add streptavidin-PE



Wash 3 x 100 µl

Resuspend in 125 µl assay buffer, shake at 850 rpm for 30 sec

Acquire data on Bio-Plex system

Read assay on instrument

RESULTS = Sample concentrations for multiple targets in < 3 hrs!

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Bio-Plex Manager 6.2

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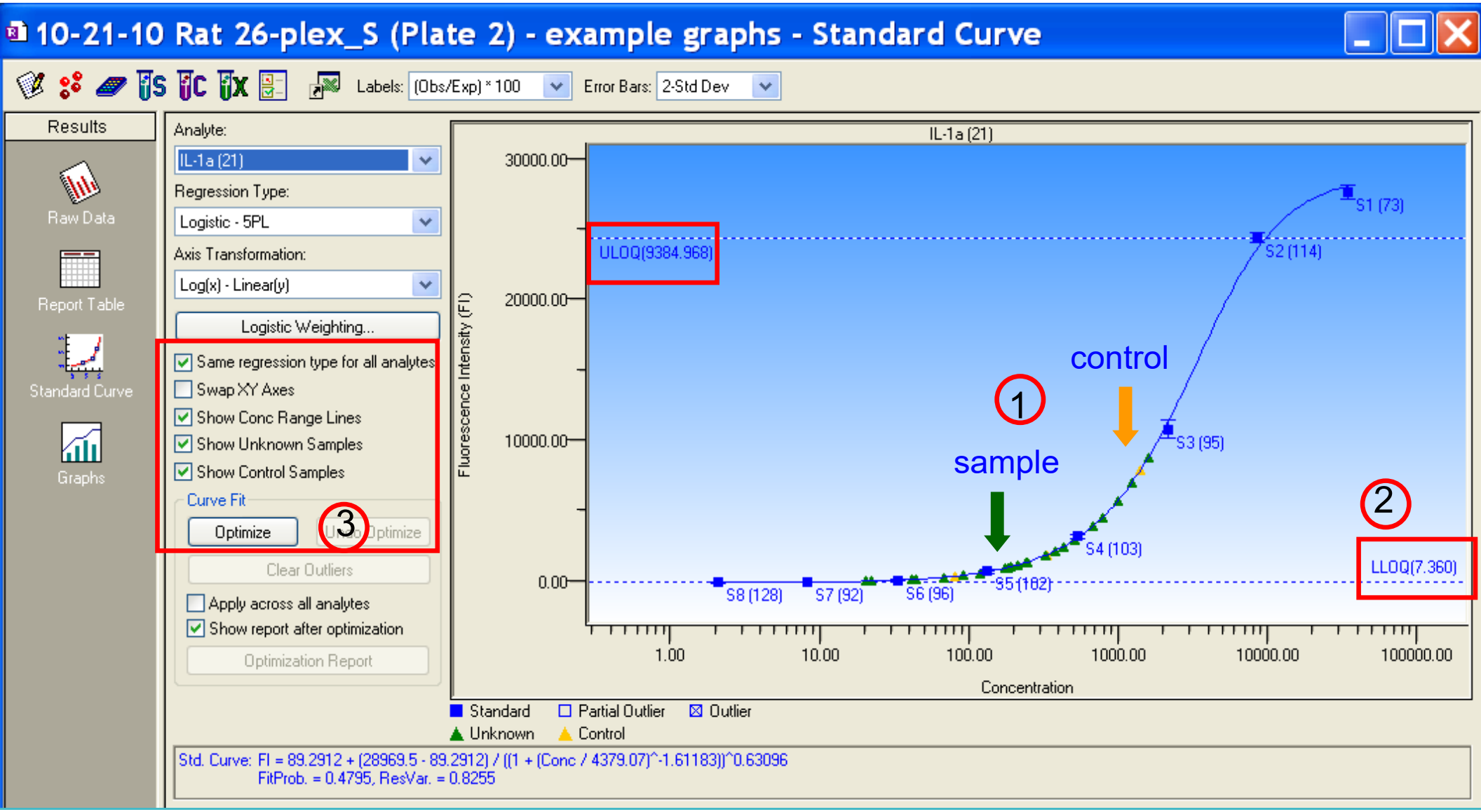
Bio-Plex Manager 6.2 Standard Curve Analysis & Graphics



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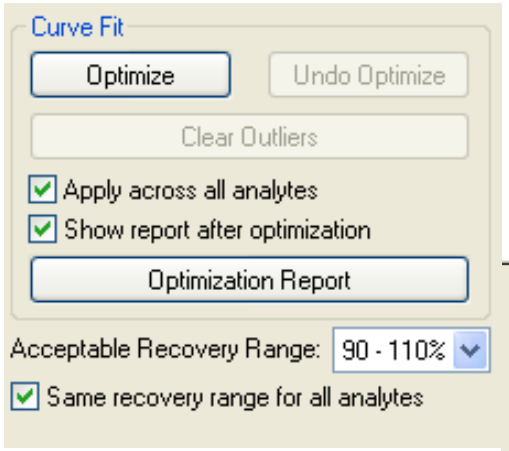
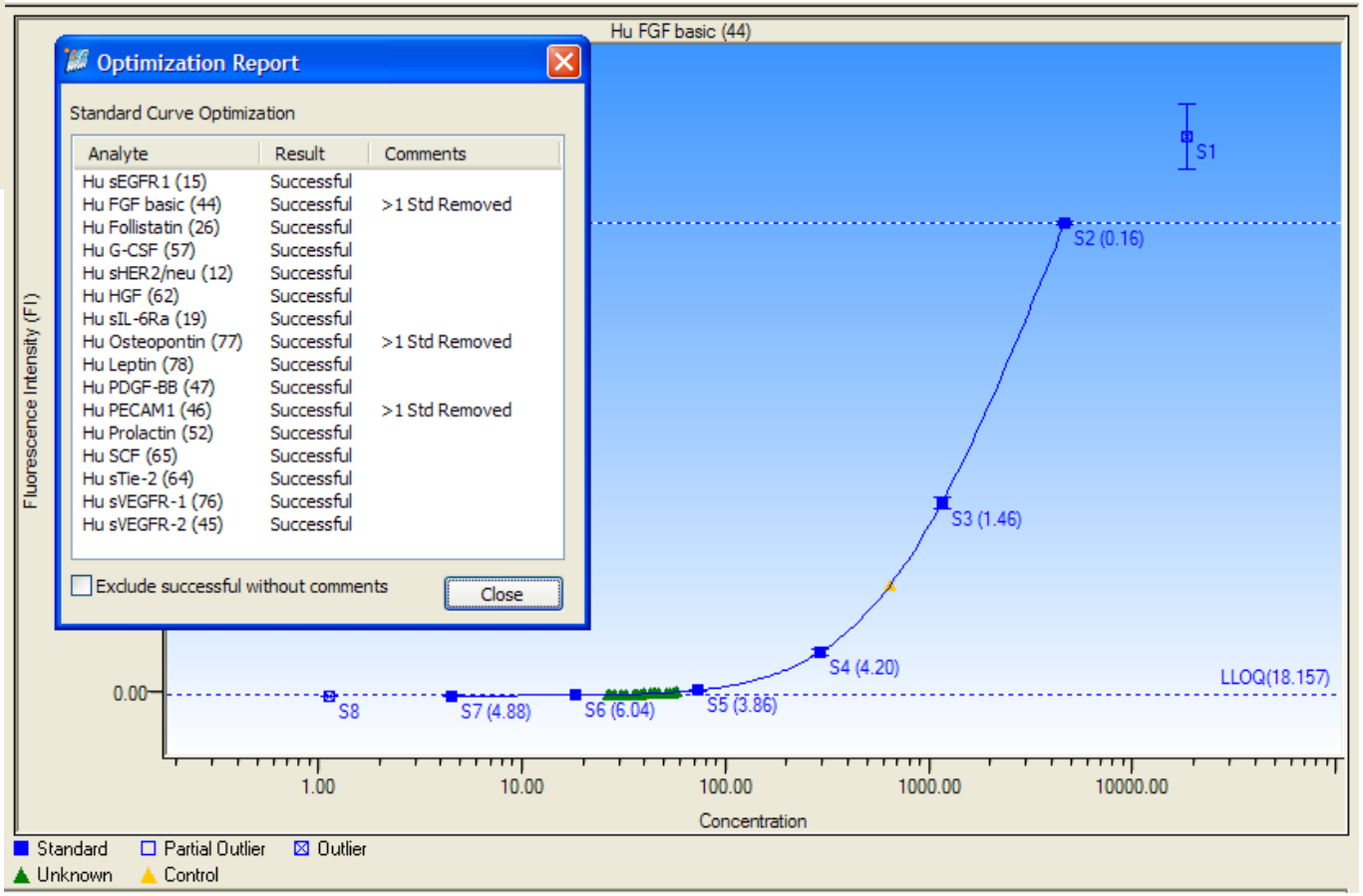
Automatic optimization with feedback





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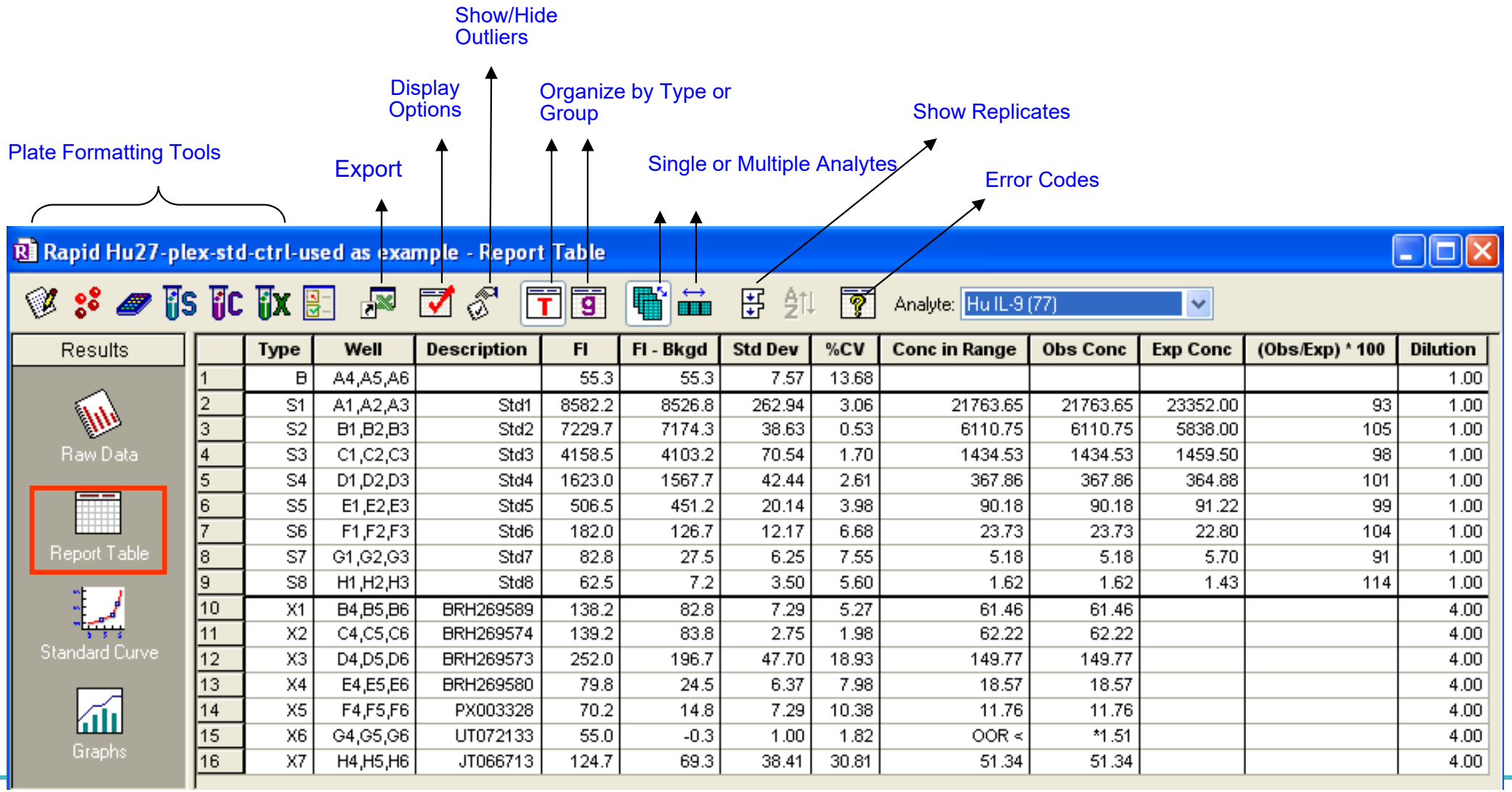
Report Table

All Relevant Results in One Customizable Table



	Type	Well	Description	FI	FI - Bkgd	Std Dev	%CV	Conc in Range	Obs Conc	Exp Conc	(Obs/Exp) * 100	Dilution
1	B	A4,A5,A6		55.3	55.3	7.57	13.68					1.00
2	S1	A1,A2,A3	Std1	8582.2	8526.8	262.94	3.06	21763.65	21763.65	23352.00	93	1.00
3	S2	B1,B2,B3	Std2	7229.7	7174.3	38.63	0.53	6110.75	6110.75	5838.00	105	1.00
4	S3	C1,C2,C3	Std3	4158.5	4103.2	70.54	1.70	1434.53	1434.53	1459.50	98	1.00
5	S4	D1,D2,D3	Std4	1623.0	1567.7	42.44	2.61	367.86	367.86	364.88	101	1.00
6	S5	E1,E2,E3	Std5	506.5	451.2	20.14	3.98	90.18	90.18	91.22	99	1.00
7	S6	F1,F2,F3	Std6	182.0	126.7	12.17	6.68	23.73	23.73	22.80	104	1.00
8	S7	G1,G2,G3	Std7	82.8	27.5	6.25	7.55	5.18	5.18	5.70	91	1.00
9	S8	H1,H2,H3	Std8	62.5	7.2	3.50	5.60	1.62	1.62	1.43	114	1.00
10	X1	B4,B5,B6	BRH269589	138.2	82.8	7.29	5.27	61.46	61.46			4.00
11	X2	C4,C5,C6	BRH269574	139.2	83.8	2.75	1.98	62.22	62.22			4.00
12	X3	D4,D5,D6	BRH269573	252.0	196.7	47.70	18.93	149.77	149.77			4.00
13	X4	E4,E5,E6	BRH269580	79.8	24.5	6.37	7.98	18.57	18.57			4.00
14	X5	F4,F5,F6	PX003328	70.2	14.8	7.29	10.38	11.76	11.76			4.00
15	X6	G4,G5,G6	UT072133	55.0	-0.3	1.00	1.82	OOR <	*1.51			4.00
16	X7	H4,H5,H6	JT066713	124.7	69.3	38.41	30.81	51.34	51.34			4.00

BIO-RAD

Bio-Rad: Premium Instrument Supplier and Service Provider

- One experienced provider for all your needs
- One call for technical support
- One partner for your success

Instrumentation

Software

- Data Pro
- Organize, automate and visualize data
- Bio-Plex Manager
  - Integrated instrument control and data analysis
  - Genotyping and pathogen detection data analysis

Bio-Plex

Assays

- Assay Builder
- Cytokines
- Disease Assays
  - Cancer
  - Diabetes
  - Isotyping
  - Acute phase
  - Angionenesis
- Signal transduction
- Assay development reagents

Service

Application Support