Making the Most of your samples with MILLIPLEX® kits

Solutions for multiplex protein detection and other high impact assays

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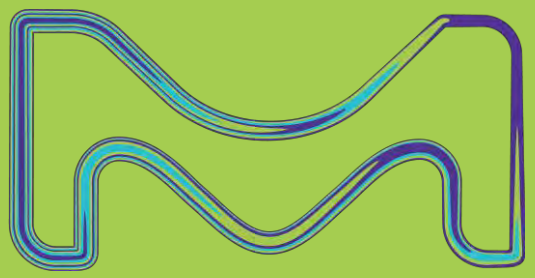
Biology Field Application Scientist
Dalhousie – May 23, 2024



EMD SERONO MilliporeSigma EMD ELECTRONICS

Taking research from idea to reality.

Model

Sample/Starting Point

- Create or collect a biologically relevant specimen
- Gain understanding of the biological system under study.
- Foundation for experimental design & further investigation.

Manipulate

Experimental Manipulation/ Sample Prep

- Aim to modify or isolate specific components to investigate their functions or properties.
- Utilize techniques such as cell culture, genetic engineering, and molecular/protein biology methods.

Measure

Analysis/ Answer

- Obtaining quantitative data and analyzing the outcomes of the experiments.
- Researchers use various techniques to measure and assess the results, depending on their specific objectives.

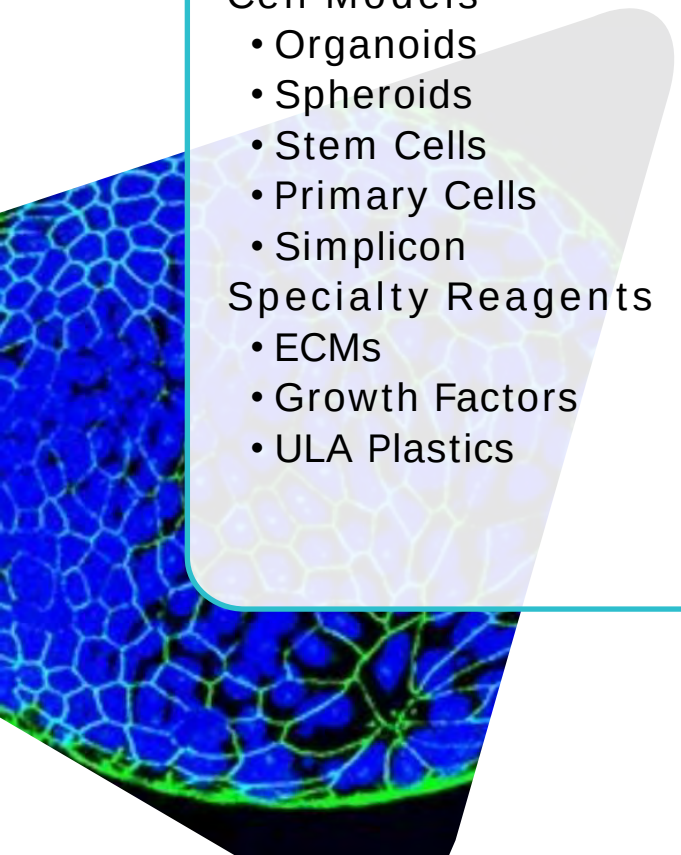
Taking research from idea to reality.

Model

Advanced Cell Culture

Cell Models
- Organoids
- Spheroids
- Stem Cells
- Primary Cells
- Simplicon

Specialty Reagents
- ECMs
- Growth Factors
- ULA Plastics

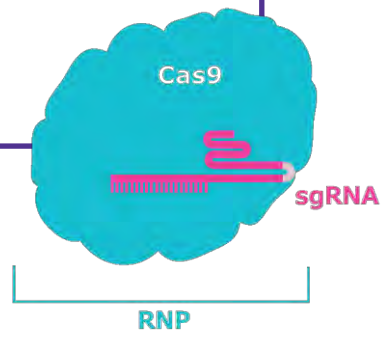


Manipulate

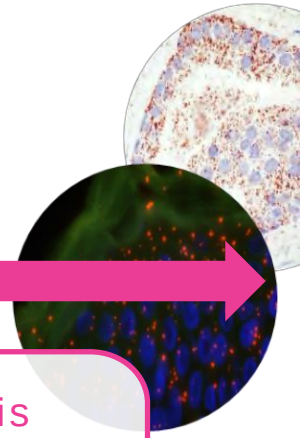
Gene Editing & Modulation

DNA Editing
- CRISPR
- Zinc Finger Nucleases (ZFNs)

RNA Modulation
- shRNA
- siRNA
- CRISPRi
- CRISPRa



Measure



Advanced Analysis

Immunoassays
- MILLIPLEX
- Westerns
- ELISA

Cell Based Assays
- Duolink PLA
- Epigenetics
- Cell Health/Death
- Live Cell Imaging

Cell Counting
- Scepter 3.0
- Millicell DCI

MILLIPLEX® Multiplex Immunoassays

History Highlights

1995

1995
Luminex
Corporation
Founded

1999 Luminex®
100™ Launched

2005 Luminex® 200™
Launched

2009 FLEXMAP 3D®
Launched

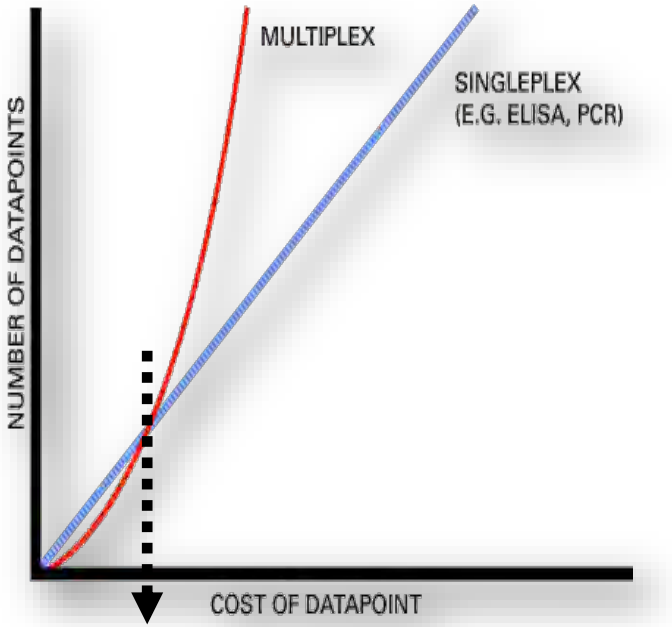
2010 MAGPIX®
Launched

2021 xMAP® INTELLIFLEX
and
xMAP® INTELLIFLEX DR-
SE Launched:
First xMAP Instrument
with Dual Reporter

2023

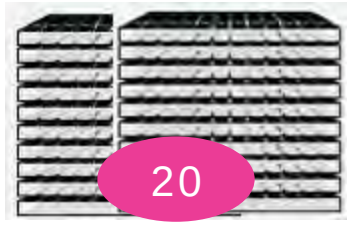
- True power of multiplex using Luminex xMAP® Technology, combining advanced fluidics, optics, and digital signal processing with proprietary microsphere ("bead") technology.
- More than 25 years of multiplexing experience.
- Demonstrated history of evolving platforms to meet changing customer needs.

Comparison of Immunoassays: Multiplex vs. ELISA



3 Proteins

For detection of 20 biomarkers in 38 samples (run in duplicate)

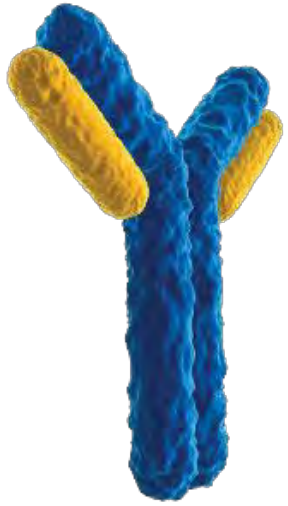
	MILLIPLEX® Panels	ELISA
Number of plates required		
Total time to result	3 hours	70 hours
Results per plate (duplicates)	1,520	76
Total volume required per sample	10-50 µL	1.5-2 mL
Dynamic range	1-10,000 pg/mL	10-2,500 pg/mL
Lower limit of detection	~1 pg/mL	~1 pg/mL

xMAP® Technology Defined

What does xMAP® mean?

x = unknown
M = multi
A = analyte
P = profiling

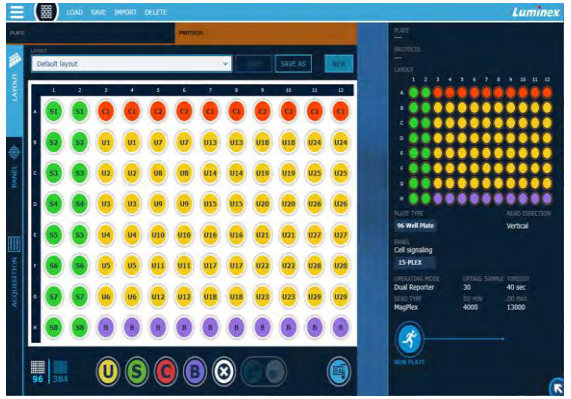
- Four Basic Components of xMAP® Technology



Biological Reagents

Microspheres

Detection Instruments

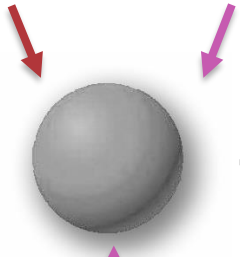


Software (Acquisition & Analysis)

Luminex xMAP® Technology
Multiple Different Analytes per Well

Luminex internally color-codes microspheres with precise concentrations of fluorescent dyes

Red Dye Infrared Dye 1



Infrared dye 2 *(FLEXMAP 3D and INTELLIFLEX Only)*

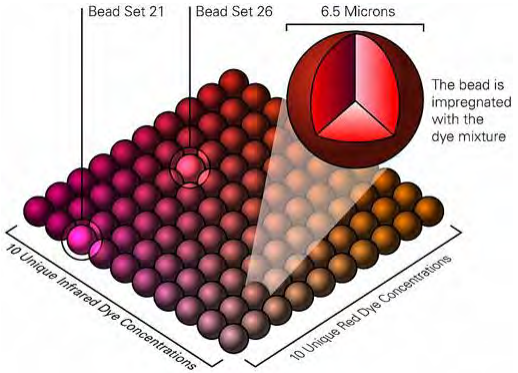
'Spectral Address' Bead set is specifically identifiable based on dye content

Each bead set has a unique ratio of dyes

LX200

FLEXMAP 3D®

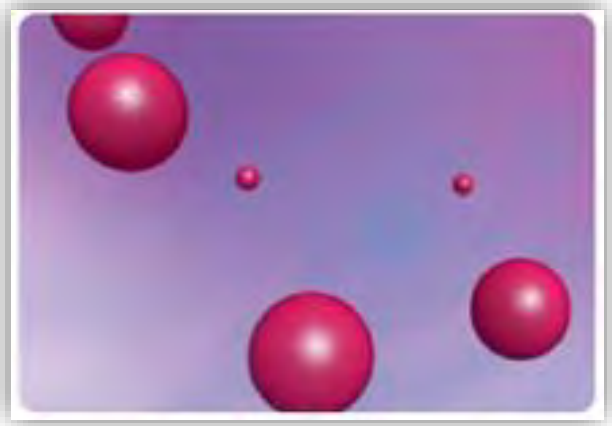
INTELLIFLEX



- Up to 100 beads/well
- 96 Well plate

- Up to 500 beads/well
- 96 & 384 Well plates

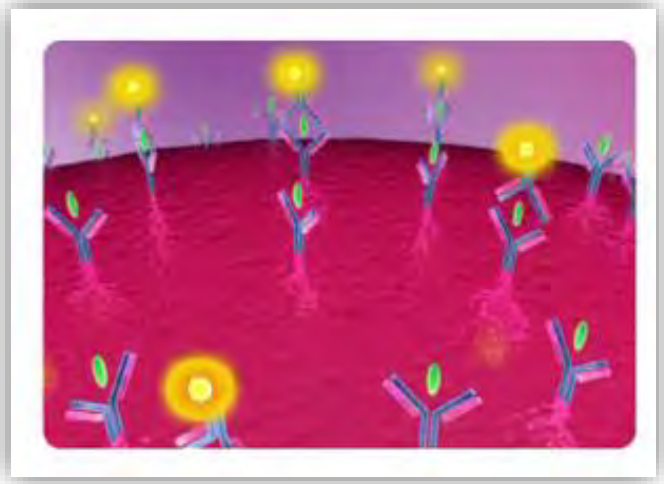
How Does xMAP® Technology Work?



Microspheres are in suspension

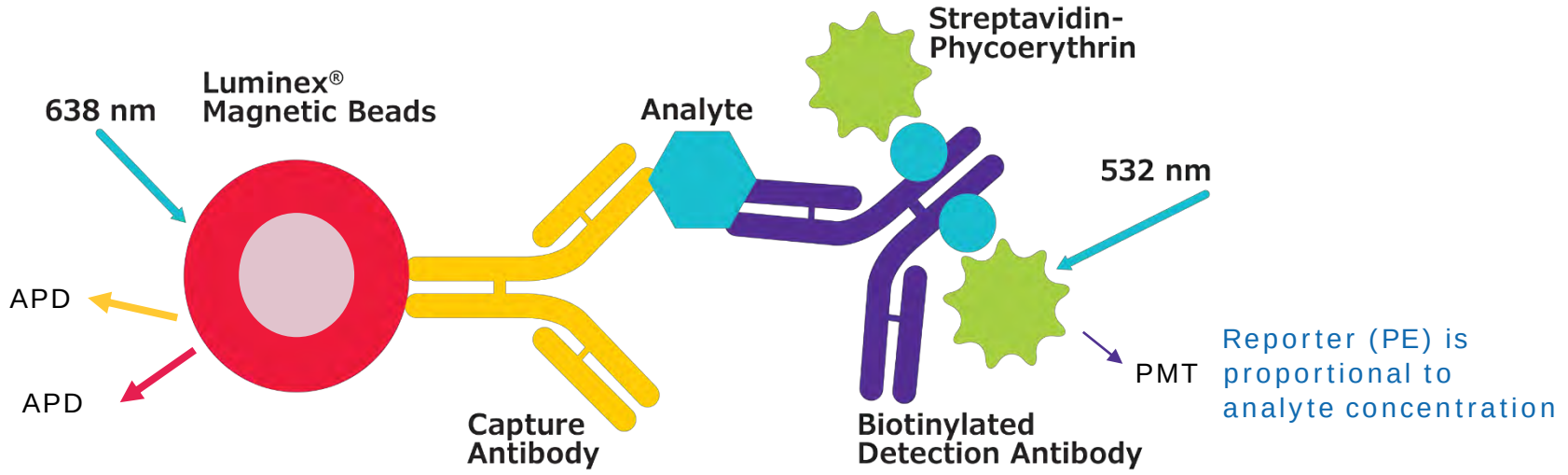
Microspheres are coated with capture antibody

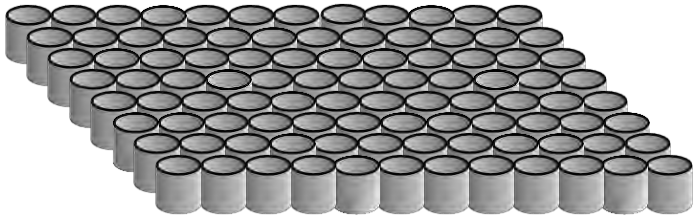
Sample is added &
Analyte is captured to microspheres



Fluorescent reporter tag added

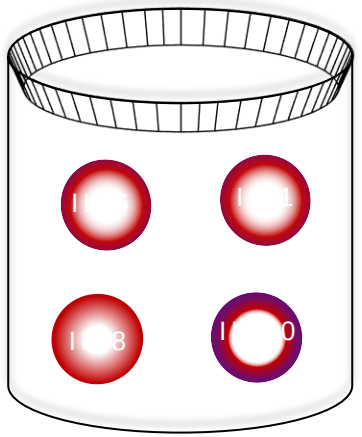
MILLIPLEX® Panels: Sandwich Complex and Multiplexed Detection





96-well plate

Multiplexing Terminology
1 Analyte = Single Plex
A Four Plex is Shown

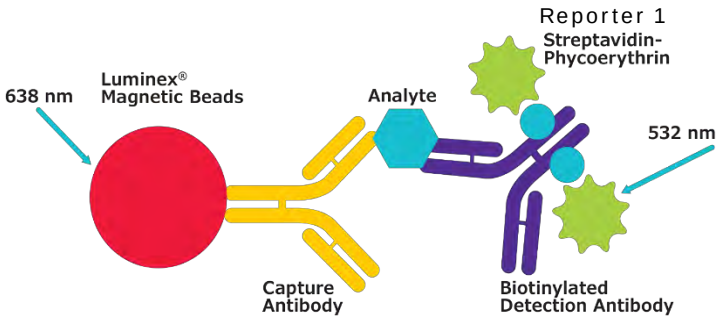


4 beads with target-specific capture antibodies conjugated to Luminex® beads

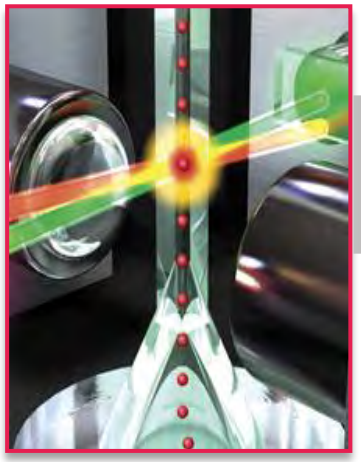
1 Bead = 1 Analyte

Detection of Analytes

Luminex® Instruments

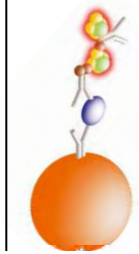


Luminex® 200™, FLEXMAP 3D®, & xMAP® INTELLIFLEX® Platforms



Green laser

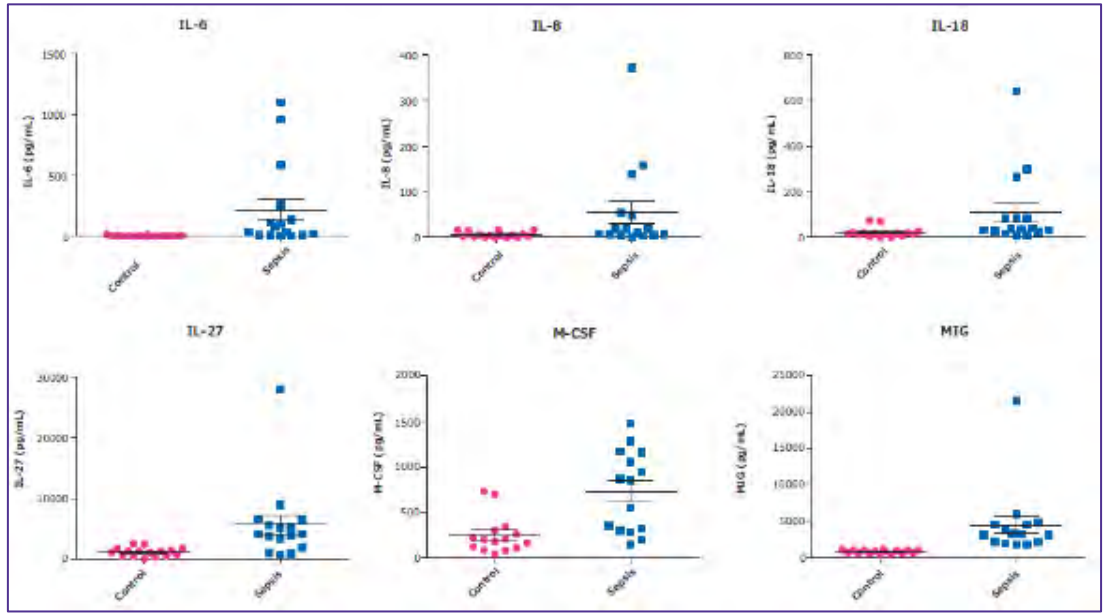
Red laser



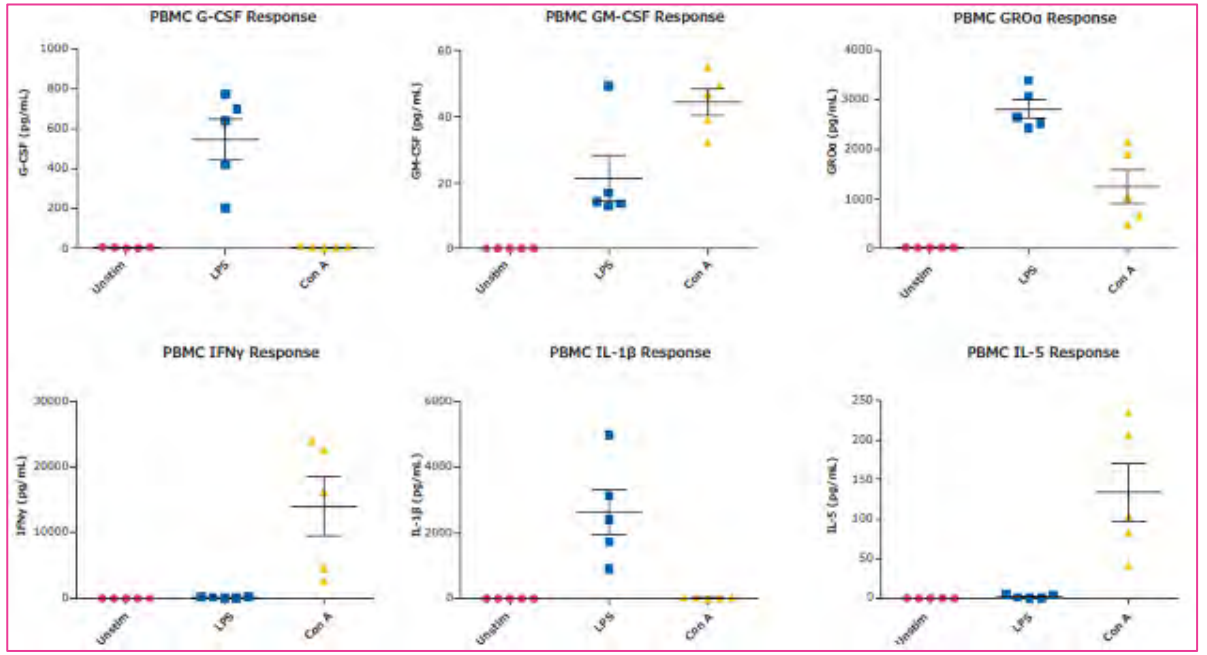
Quantify assay reporter dye

Identify bead color for analyte identification

Human Cytokine/Chemokine/Growth Factor Panel A
What does Milliplex data look like?



Stimulated PBMCs vs. Control



Serum/Plasma Samples:
Sepsis vs. Healthy

Taking research from idea to reality: A milliplex use case scenario

Model

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Measure

Analysis/ Answer

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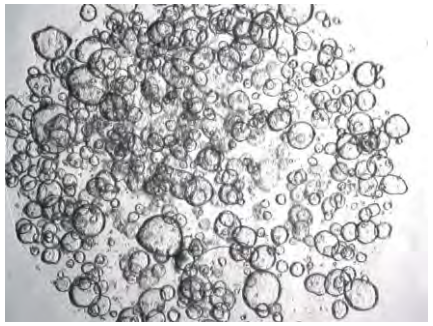
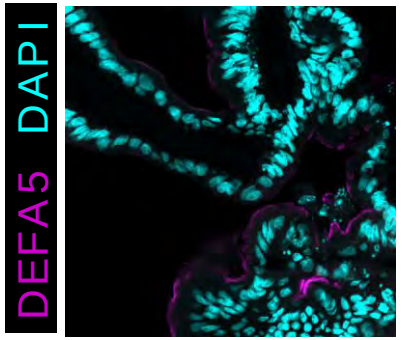
Taking research from idea to reality: A milliplex use case scenario

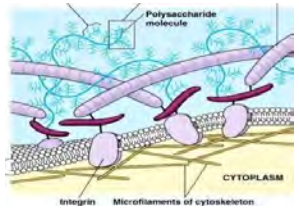
Model

Normal Duodenum

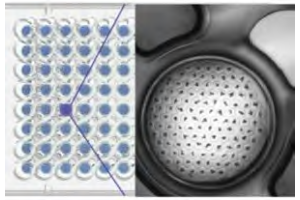




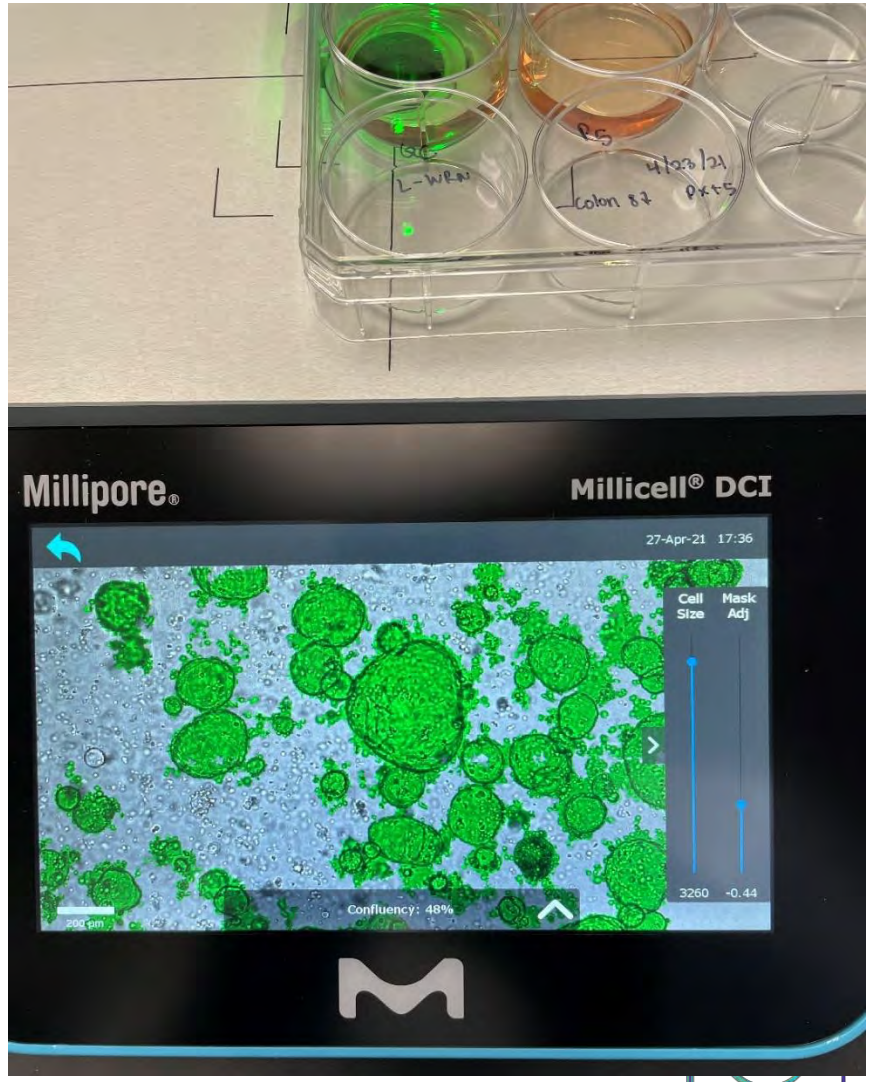
3dGRO™ Wnt3a Conditioned Media Supplement



ECM Gel from Engelbreth-Holm-Swarm murine sarcoma



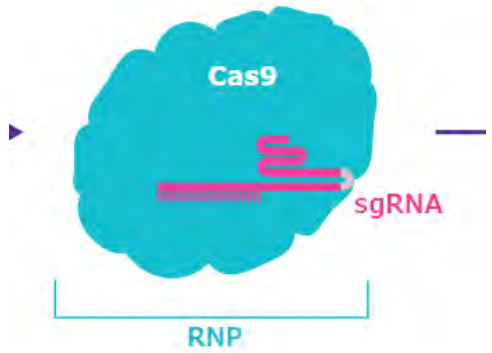
Millicell® Microwell Plates



Taking research from idea to reality: A milliplex use case scenario

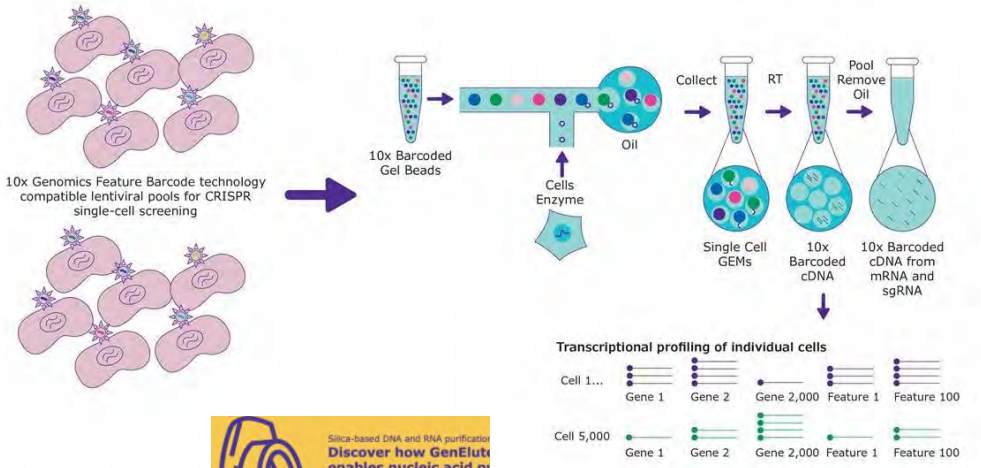
- **CRISPR – knockout, knock-in, nickase, inhibition, activation**
- **RNAi: shRNA, siRNA, esiRNA**
- **Overexpression ORFs**
- ZFNs
- miRNA mimics and inhibitors
- PROTAC® Degraders
- Growth Factors and Cytokines
- Bioactive Small Molecules
- Cell Cycle Bioactive Small Molecules
- Bioactive Small Molecules for Kinase Phosphatase Biology
- Bioactive Small Molecules for Gene Regulation
- Bioactive Small Molecules for Apoptosis

Manipulate



PEXBUFF Transfection enhancer

10x Compatible CRISPR

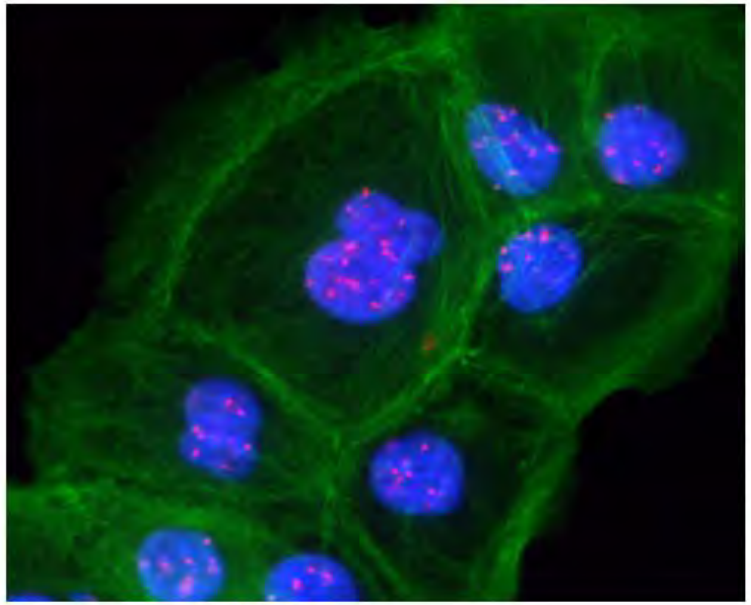


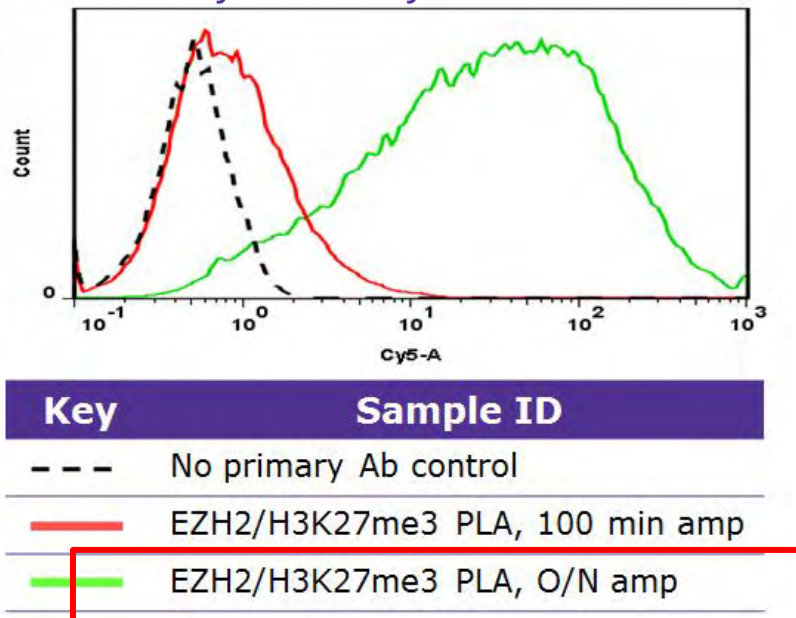
GenElute-E

Novel lysis allows negative chromatography to be used for high quality NAP

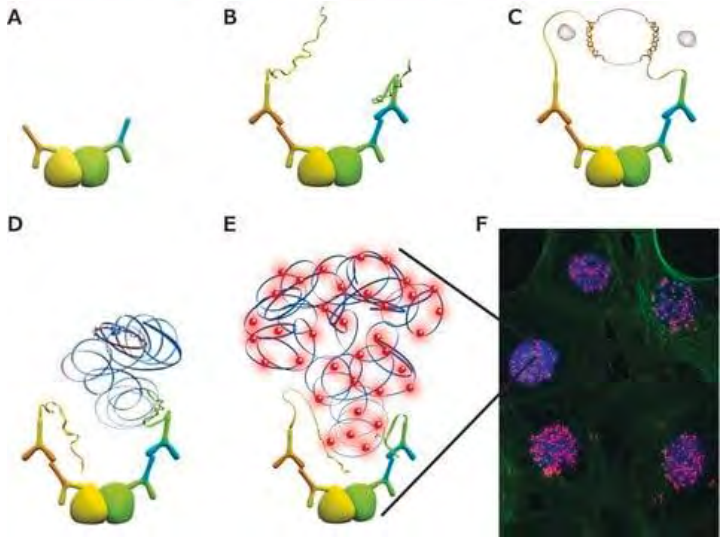
Taking research from idea to reality: A milliplex use case scenario

Duolink PLA with Flow Cytometry





Measure



Duolink Proximity Ligation Assay (PLA)

Milliplex Immunoassay

NEW! MILLIPLEX® PLEXpedition 115-plex Screening Panel

Versatile solution designed for diverse research areas

xMAP® INTELLIFLEX® or FLEXMAP 3D® Instrument

Luminex® 200™ Instrument

MAGPIX® Instrument

EGF	IL-4	MIG	ACTH	M-CSF	Amylin (Active)	MCP-4
FGF-2	IL-5	MIP-1α	BDNF	MCP-3	BCA-1	MIP-1δ
Fractalkine	IL-6	MIP-1β	C-Peptide	MDC	BNP	MIP-3β
G-CSF	IL-7	OPN	Eotaxin	MIP-3α	CK-MB	MMP-3
Ghrelin (Active)	IL-8	PDGF-AA	FABP4	MMP-1	GCP-2	MMP-10
GIP	IL-10	PDGF-BB	FLT3 Ligand	MMP-2	DKK1	MMP-12
Glucagon	IL-12 (p40)	Perforin	GLP-1 Total	MMP-7	ENA-78	Myostatin
GM-CSF	IL-12 (p70)	RANTES	Granzyme A	MMP-9	Eotaxin-2	NGF
Granzyme B	IL-13	sCD40L	I-TAC	OPG	Eotaxin-3	NTproBNP
GROα	IL-15	SCF	IL-3	PIGF	Erythropoietin (EPO)	Osteocalcin
HGF	IL-17A	sFAS	IL-9	PP	FABP3	Osteonectin
IFNα2	IL-18	sFASL	IL-17F	PYY	FGF-21	SDF-1
IFNγ	IL-22	TGFα	IL-21	TARC	FGF-23	SOST
IL-1α	IL-23	TNFα	IL-33	TNFβ	HB-EGF	TPO
IL-1β	IP-10	TRAIL	Insulin	Troponin I	I-309	TSLP
IL-1Ra	Leptin	VEGF-A			IL-11	VEGF-C
IL-2	MCP-1				LIF	VEGF-D
					MCP-2	

Detect markers spanning multiple research areas:

- Inflammation / Immunology Markers
- Metabolism Endocrinology Markers
- Cancer Markers
- Cardiovascular Disease Markers
- Bone Markers
- Neuroscience Markers
- Toxicity Markers

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Versatile solution designed for diverse research areas

xMAP® INTELLIFLEX® or FLEXMAP 3D® Instrument

Luminex® 200™ Instrument

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EGF	IL-4	MIG	ACTH	M-CSF	Amylin (Active)	MCP-4
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G-CSF	IL-7	OPN	Eotaxin	MIP-3α	CK-MB	MMP-3
Ghrelin (Active)	IL-8	PDGF-AA	FABP4	MMP-1	GCP-2	MMP-10
GIP	IL-10	PDGF-BB	FLT3 Ligand	MMP-2	DKK1	MMP-12
Glucagon	IL-12 (p40)	Perforin	GLP-1 Total	MMP-7	ENA-78	Myostatin
GM-CSF	IL-12 (p70)	RANTES	Granzyme A	MMP-9	Eotaxin-2	NGF
Granzyme B	IL-13	sCD40L	I-TAC	OPG	Eotaxin-3	NTproBNP
GROα	IL-15	SCF	IL-3	PIGF	Erythropoietin (EPO)	Osteocalcin
HGF	IL-17A	sFAS	IL-9	PP	FABP3	Osteonectin
IFNα2	IL-18	sFASL	IL-17F	PYY	FGF-21	SDF-1
IFNγ	IL-22	TGFα	IL-21	TARC	FGF-23	SOST
IL-1α	IL-23	TNFα	IL-33	TNFβ	HB-EGF	TPO
IL-1β	IP-10	TRAIL	Insulin	Troponin I	I-309	TSLP
IL-1Ra	Leptin	VEGF-A			IL-11	VEGF-C
IL-2	MCP-1				LIF	VEGF-D
					MCP-2	

Three options available:

- 115-plex configurable Panel HPLX1-115SP
- 115-plex fixed analyte kit HPLX1-115SP-PX
- 80-plex fixed analyte kit HPLX1-115SP-PX80

- Includes all necessary components: premixed beads and detection antibodies
- Utilizes critical raw materials from MILLIPLEX® Qualified Assays, ensuring research continuity
- Sample agnostic compatible with various sample types, offering flexibility
- Saves sample, time and money by enabling detection of 115 proteins at one time

NEW* Cell Signaling

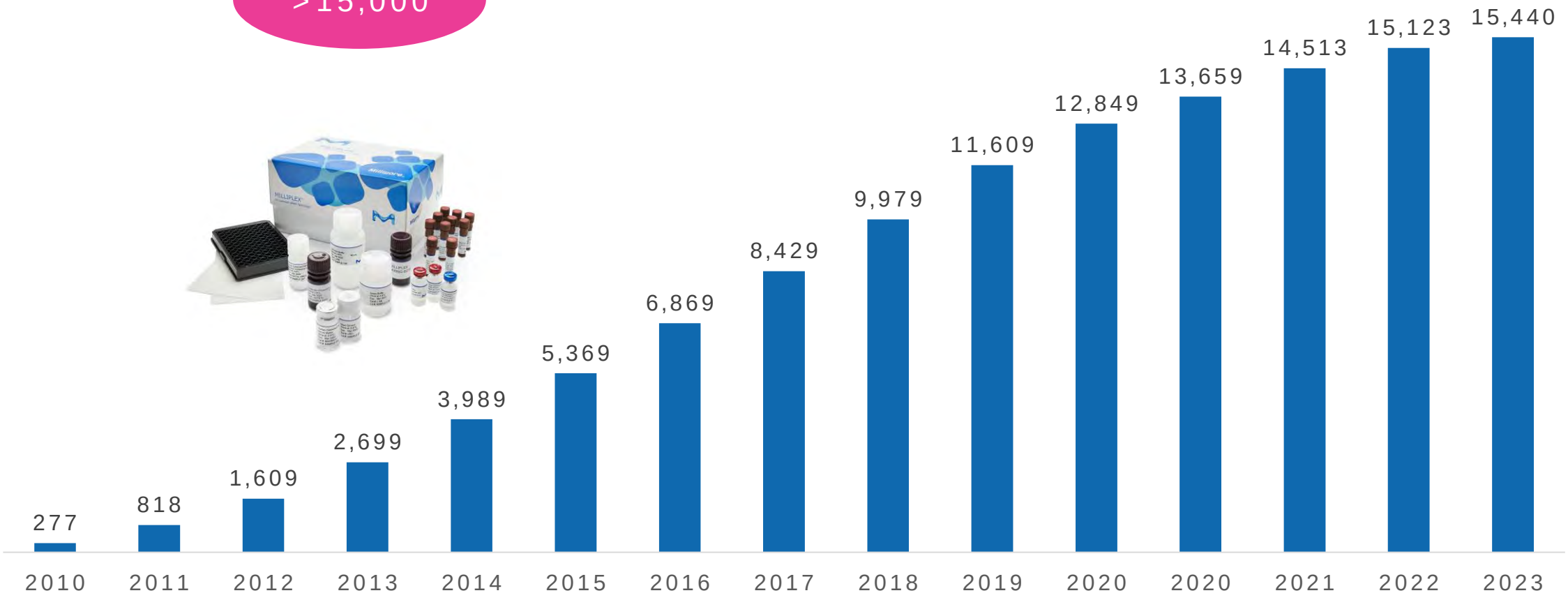
MILLIPLEX® Human Cell Health Magnetic Bead Panel

Cell health panel is a fixed 16 plex assay that provides understanding of the overall cell health. Each of the analyte is a marker for a particular cellular function.

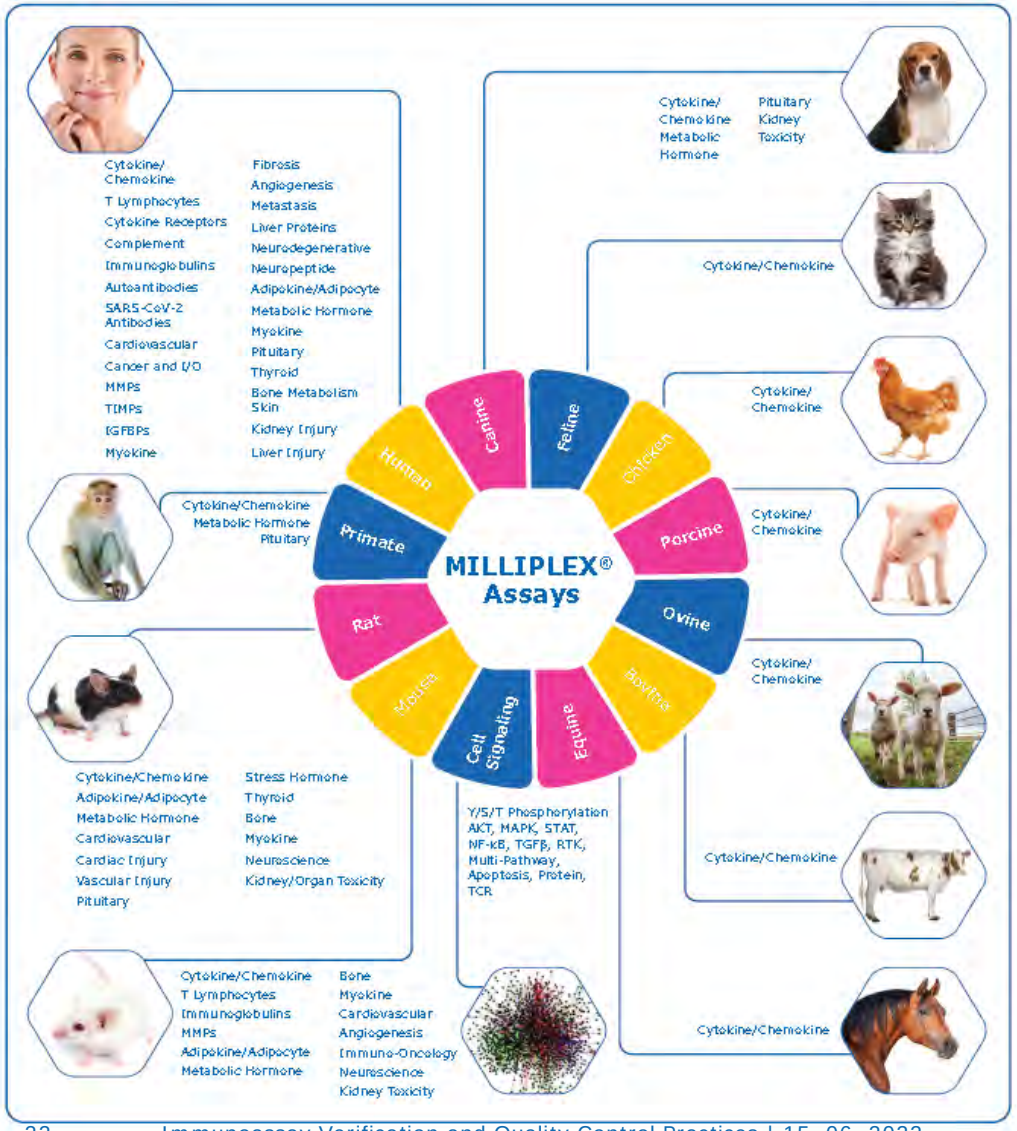
Analyte/Biomarker	Cellular Function
Histone H3 (Ser10)	Chromatin condensation
RNA Pol II (Ser2)	mRNA synthesis
Cleaved PARP	Apoptosis
Phosphorylated eIF4B (Ser422)	Protein synthesis
Complex IV	OXPHOS
Polyubiquitin K48-linkage	Protein degradation
LC3B	Autophagy
HIF-1a	Oxidative stress
HSP70	General stress
Histone H2A.x (Ser139)	DNA repair
CHOP	Protein secretion
Cyclin B1	Cell cycle (G1/S)
GRP78	ER stress
p53 (Ser15)	DNA damage
NF-κB (Ser536)	Inflammatory Response
Ki-67	Cell Viability and Proliferation

Why Use Milliplex kits?
Scientific Discoveries: MILLIPLEX® Assay Publications

>15,000



Why use Milliplex kits?
Explore the Extensive Selection of MILLIPLEX® Panels



+225 MILLIPLEX® Panels
+1,100 analytes

covering a broad range of species and research areas

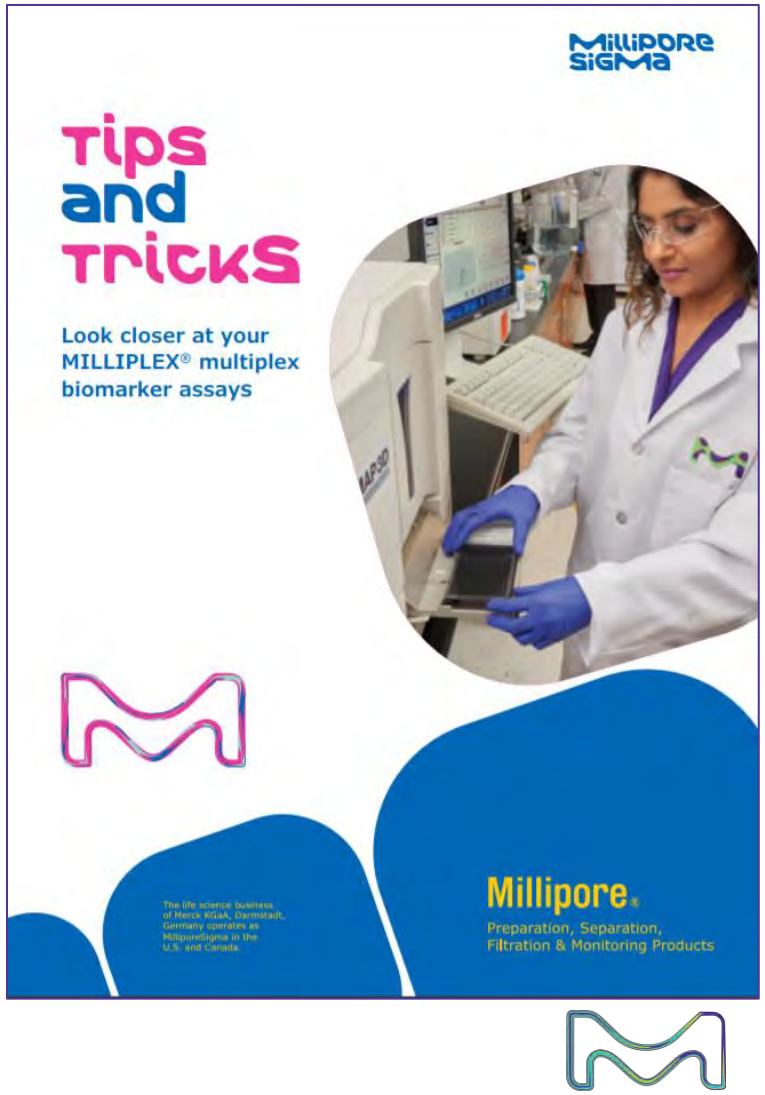
Why Choose our MILLIPLEX® Panels?

- Configurable to Suit Your Research Needs
- Convenient Premixed Options
- Available in Bulk Kits for High-Volume Requirements
- Pre-Optimized Kit Components for Easy Use and Minimal Preparation Steps

MILLIPLEX® Multiplexing Assays

Samples types

Serum/plasma Cell culture Supernatants Cell lysates	Cerebrospinal fluid Synovial Fluid	Tissue homogenates gut, lung, brain, skin, tumor, muscle…
Eluted Blood Spots Breath condensate	**Known Sample Types**	Nasal wash Tears, aqueous humors
Urine Sputum	Vaginal/Cervical Wash Extracted Cervical Secretions	Bronchial Alveolar Lavage Fluid



Our MILLIPLEX® Kit!

- Each MILLIPLEX® kit includes all necessary reagents to prepare the assay to read on your Luminex® instrument, just add samples and water

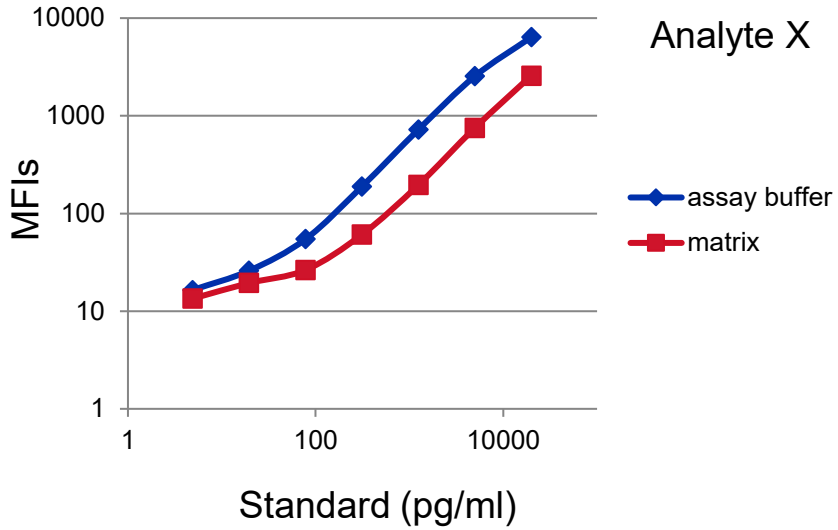
Key differentiators

**Components differ in MILLIPLEX® Autoantibody kits and Cell Signaling kits*

Need for Optimized Matrix in the Standard Curve

Standard Curve MFIs

Standard (pg/ml)	Assay Buffer	Serum Matrix
0	11	5
5	17	14
20	26	20
78	55	27
313	190	61
1250	725	196
5000	2549	751
20000	6409	2574



Average Serum Sample Recovery

Assay Recovery	IFNγ	IL-1β	TNFα	VEGF-A
Against Buffer Curve	34%	40%	29%	39%
Against Matrix Curve	98%	94.9%	97%	91%

Serum Matrix Effects

Average Serum Sample Recovery for Diluted Sample against Buffer Standard Curve

Human Cytokine Magnetic Panel I

Sample Dilution	Eotaxin	GM-CSF	IFNγ	IL-1β	IL-6	MCP-1	TNFα	VEGF
Neat	71%	48%	34%	40%	60%	61%	29%	39%
Diln 1:4	69%	68%	49%	63%	78%	63%	52%	51%
Diln 1:10	72%	67%	61%	74%	81%	69%	64%	63%
Diln 1:20	77%	77%	69%	81%	86%	75%	75%	73%

Average Serum Sample Recovery against Serum Matrix Standard Curve

Human Cytokine Magnetic Panel I

Sample Dilution	Eotaxin	GM-CSF	IFNγ	IL-1β	IL-6	MCP-1	TNFα	VEGF
Neat	100.5%	100.7%	98.1%	94.9%	96.1%	98.3%	97.8%	91.8%

The MILLIPLEX® Panels: Genuine PlexAbility

Human Cytokine/Chemokine/ Growth Factor Panel A

(Cat. No. HCYTA-60K)
(Cat. No. HCYTA-60K-PX38)♦
(Cat. No. HCYTA-60K-PXBK38)♦
(Cat. No. HCYTA-60K-PX48)
(Cat. No. HCYTA-60K-PXBK48)

sCD40L	IL-13♦
EGF♦	IL-15♦
Eotaxin/CCL11♦	IL-17A/CTLA8♦
FGF-2/FGF-basic	IL-17E/IL-25♦
Flt3 Ligand	IL-17F♦
Fractalkine/CX3CL1	IL-18♦
G-CSF♦	IL-22♦
GM-CSF♦	IL-27
GROα	IP-10/CXCL10♦
IFNα2♦	MCP-1/CCL2♦
IFNγ♦	MCP-3/CCL7
IL-1α♦	M-CSF♦
IL-1β♦	MDC/CCL22
IL-1RA♦	MIG/CXCL9♦
IL-2♦	MIP-1α/CCL3♦
IL-3♦	MIP-1β/CCL4♦
IL-4♦	PDGF-AA♦
IL-5♦	PDGF-AB/BB♦
IL-6♦	RANTES/CCL5♦▲
IL-7♦	TGFα
IL-8/CXCL8♦	TNFα♦
IL-9	TNFβ/Lymphotoxin-α (LTA)♦
IL-10♦	VEGF-A♦
IL-12 (p40)♦	
IL-12 (p70)♦	

ONE Assay: MANY Configurations

Potential Assays for an HCYTA-60K 5 Plex

IL-2	IL-7	IP-10
IL-4	IL-13	TNFα
IL-5	IL-15	IL-17F
IL-6	IL-22	MIP-1α
IL-10	IL-27	IL-1β

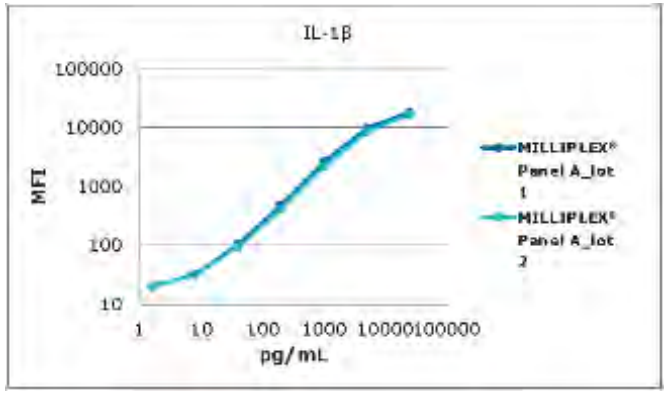
Same Base Kit: Different 1º Beads

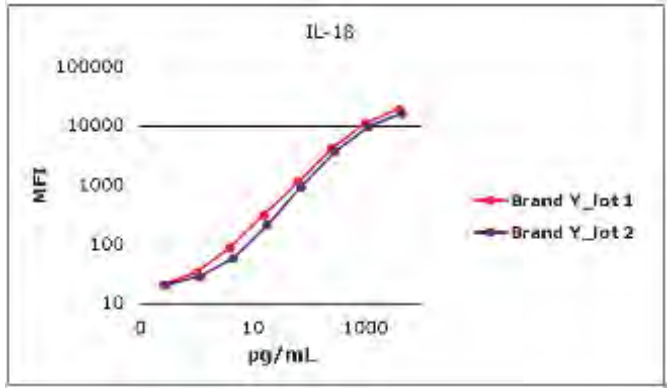
There is NO performance change with configuration

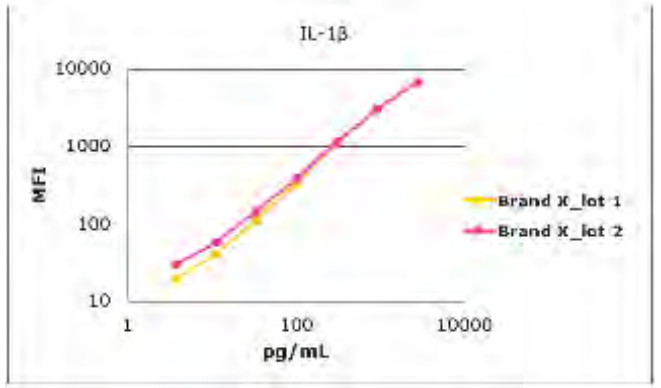
27

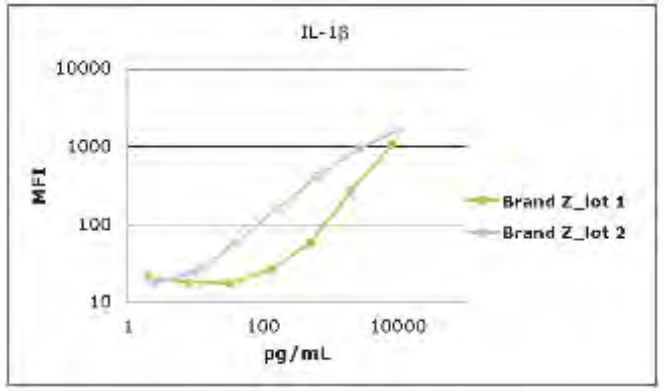
Why use Milliplex kits?
Superior Lot-to-Lot Performance Demonstrated In MILLIPLEX® Kits

- Standard curves were generated from each kit lot using averaged mean fluorescence intensity (MFI) from duplicate wells
- MILLIPLEX® kits maintain a consistent standard curve from lot-to-lot
- Other suppliers value-assign their standards
  - Each lot has a different standard curve range for each analyte









Illuminate your assay performance
Belysa Immunoassay curve fitting software

- Belysa is a curve fitting software that will analyze data files generated on any Luminex instrument
- Software is compatible with 96/384 well assays
- Belysa can also be used with ELISA and other immunoassays such as our SMC (single molecule counting) platform

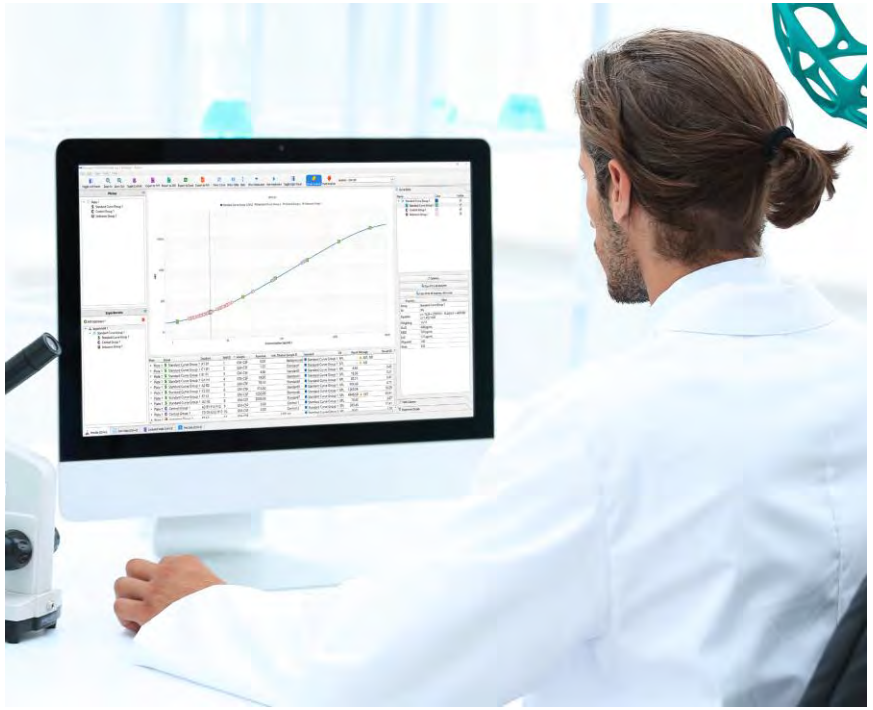
Luminex instruments: MagPix, LX200, Flexmap 3D, Intelliflex

- The user-friendly software has been designed to quickly analyze your single or multi-analyte experiment using advanced curve fit and data optimization features. Resulting in accurate, reliable reporting.

Belysa Immunoassay curve fitting software

Features:

1. Curve fitting (4pl, 5pl, linear, competitive, cubic spline
2. Rules-based data flagging
3. Curve comparison tools
   - Parallelism coefficient to compare curves between plates
4. User friendly with drag and drop interface for xPONENT files
5. Curve optimization wizard
6. Suitable for use with Luminex, SMCxPRO, and ELISA data
7. Compatible with Windows and macOS



Intuitive, user-friendly curve fitting for better immunoassay data!!!

Discover with Confidence

Benefits of Our Biomarker Kits

Consistency & Reproducibility: We ensure lot-to-lot consistency with our biomarker kits that perform at the individual assay level as well as over time for reliably reproducible results.

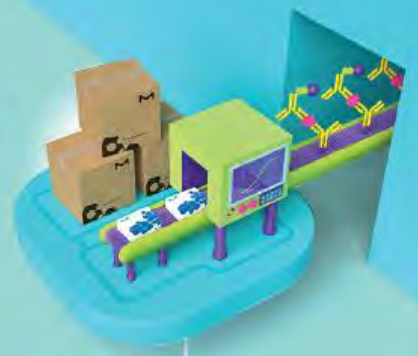
Verification: Our biomarker kits are rigorously tested with a range of verified methods to ensure reliable performance.

Simplicity: To eliminate the added potential for error, all our biomarker kits come with plug-and-play materials, repeatable step-by-step protocols, and dedicated expert support.

Sensitivity: When your research requires the detection of low-abundant biomarkers or biologically relevant samples, our biomarker kits offer the sensitivity you need to achieve your goals.

Beyond Biomarkers

We are committed to delivering the best possible immunoassays for your research needs. Whether you are using our industry-leading MILLIPLEX® multiplex panels to broadly survey multiple analytes, performing femtogram/mL biomarker analysis using the SMC® ultrasensitive immunoassay platform, or examining single proteins with our tried-and-true ELISAs and RIAs, you can confidently expect reliable measurements, a simplified user experience, and knowledgeable scientific partners to help drive your biomarker project from hypothesis to publication.



Designed RelyAbility

Our Conferma® ELISAs are engineered for endogenous detection and lot-to-lot consistency of routine-use biomarkers to keep your data reproducible, run after run.

Genuine FlexAbility

Our industry-leading MILLIPLEX® multiplex assays for the Luminex® platform give you the FlexAbility to select your combination of biomarkers to simultaneously analyze with ease.

Next-Level DetectAbility

Our ultrasensitive SMC® kits for the SMCxPRO® platform take your research to the next level by detecting biomarkers down to fg/mL levels.

Complete CompareAbility

Our Belysa® Immunoassay Curve Fitting Software helps you quickly compare your standard and sample curves with ease, confirming that your methods and plates ran consistently.

Expert CustomAbility

Our Custom Assay Development & Innovation (CADI) team of experienced scientists partner with you to develop custom immunoassays for your biomarker(s) of interest, accelerating your projects from discovery to clinical trials.

Keep your focus on the next stage.

The Analyte Quarterly

New Kits – PLEXpedition, Cell Health panel, Human Exosome Characterization panel

Immunoassays at a Glance

		MILLIPLEX® Multiplex Assays			
		Qualified Assays	Screening Assays	SMC® Assays	ELISAs
Species	Human	+	+	+	+
	Mouse	+		+	+
	Rat	+		+	+
	Non-Human Primate	+		+	+
	Canine	+			+
	Feline	+			+
	Bovine	+			+
	Chicken	+			
	Equine	+			+
	Ovine	+			+
	Porcine	+			+
	Multi-Species	+		+	+
Analytes	Human	>580	115	>40	>1,000
	Mouse	>180		3	>300
	Rat	>90		3	>190
	Non-Human Primate	>60		1	>70
	Canine	>30		1	>30
	Feline	>15			>20
	Bovine	>15			>20
	Chicken	>10			
	Equine	>20			>20
	Ovine	>10			>5
	Porcine	>10			>30
	Multi-Species	>5		1	>5

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