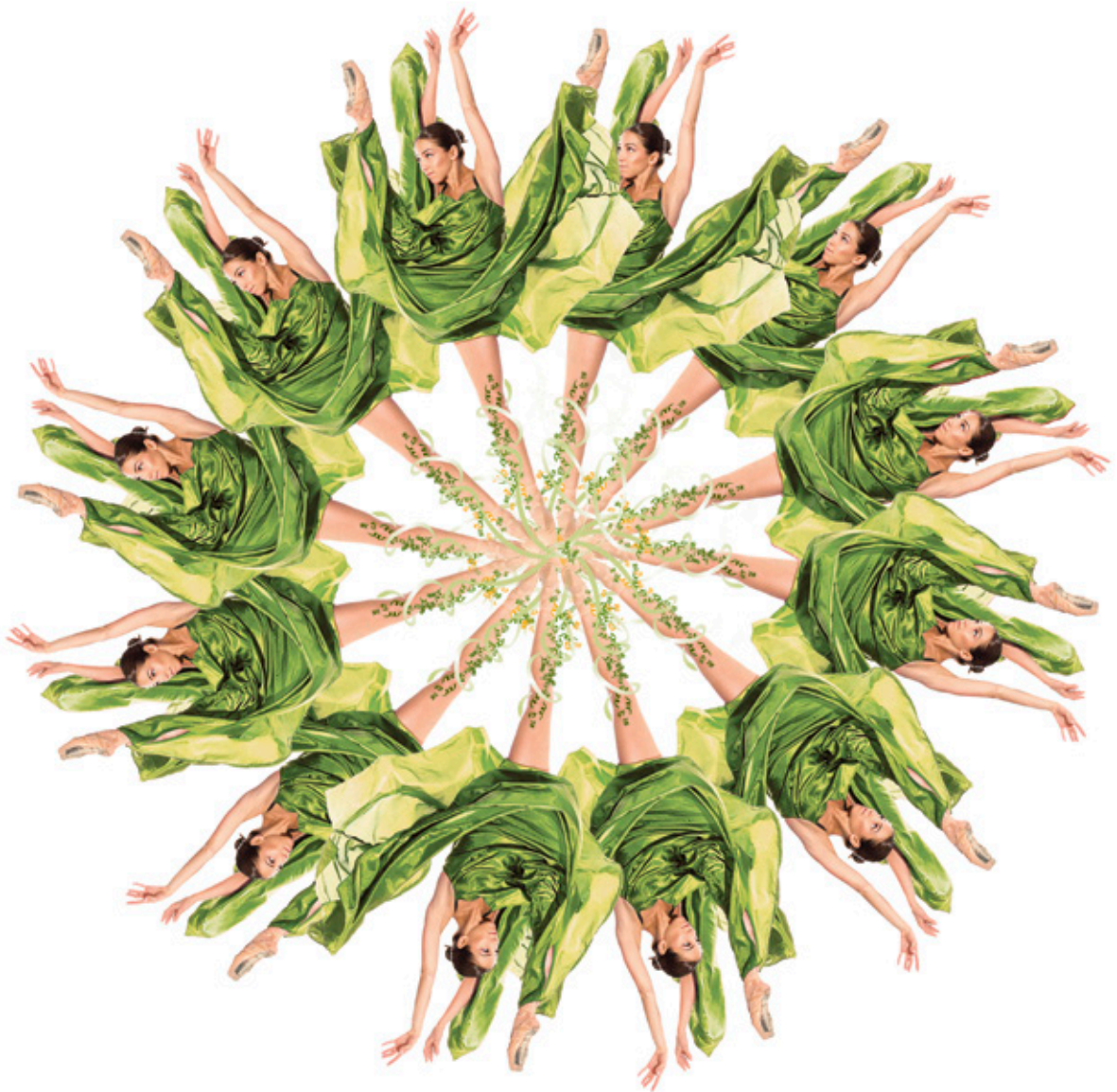


A closer look at immunoassay platforms.

# Beyond fit for purpose.

Bring your biomarkers to life with Merck Millipore Immunoassay Platform Solutions.



# As we grow to meet your research needs ...



Growing capabilities. Founded in 1978 as Linco Research, Merck Millipore's biomarker assay team has stayed true to its small-company, customer-centric values and Midwestern-U.S. roots—now backed by the resources of a global company, Merck KGaA of Darmstadt, Germany.

## ... our promise remains.

# How to choose an immunoassay platform that's beyond "fit for purpose"

What's better than a well-validated, sensitive, reproducible, robust immunoassay that fits your study requirements? It's a scientific partner offering you the choice of a variety of assay platforms, all with the same high performance.

Whether you need ultrasensitive detection from a Singulex<sup>®</sup> assay, maximum throughput from a Gyrolab<sup>®</sup> workstation, robust ELISAs or rich profiling data from a multiplex panel using Luminex<sup>®</sup> technology, we help you get data you trust from each sample, with minimal repetition, so you can make the best next-step decisions.

Get started now! We'll help you choose the right platform and assays not only to meet your needs today, but we'll partner with you to meet those needs in the future—without compromising on quality.

Luminex<sup>®</sup> platform



Multiplex detection

Singulex Erenna<sup>®</sup> system



Ultrasensitivity

ELISA



Widely cited

Gyrolab<sup>®</sup> platform







High precision

## 1. First, decide on your assay parameters:

- Multiplex capability: Plex size needed \_\_\_\_\_
- Expected concentration range \_\_\_\_\_
- Precision (inter- or intra-assay coefficient of variation %CV) \_\_\_\_\_
- Number of samples per assay (throughput) \_\_\_\_\_
- Sample volume available \_\_\_\_\_

## 2. Explore our platforms on the following pages of this brochure and on: www.merckmillipore.com/biomarkerassays

Protein Detection Platforms	Fit for Purpose	Quantitative	Sensitivity	Sample Volume	Dynamic Range	Multiplex Capability	Custom Assay Support
Luminex® platform 	Multiplex detection Flexible platform	Yes	pg	~25 µL	●●●	✓	Yes
Singulex Erenna® system 	Ultrasensitivity High performance	Yes	fg	5-100 µL	●●●	✗	Yes
ELISA 	Most widely cited Plate reader compatibility	Yes	pg	50-100 µL	●	✗	Yes
Gyrolab® work-station 	High precision Fully automated	Yes	pg	< 5 µL	●●●	✗	Yes

● Good Performance   ●● Strong Performance   ●●● Superior Performance   ✗ Not recommended   ✓ Recommended

## 3. Design your MILLIPLEX® multiplex panel, or simply order your single-protein assay

We have the largest portfolio of kits, analytes and species compared to all other commercial suppliers.

### With our combined platforms, we have:

- 5 platforms
- 285 kits to study circulating proteins
- 425 unique circulating analytes (not counting different species)
- 89 kits to study intracellular proteins
- 125 intracellular analytes
- 8 species

### A broad portfolio means that you will:

- Likely find assays for analytes that you need
- Achieve greater consistency by purchasing assays from one vendor
- Retain the flexibility to meet your needs now and in the future
  - Translational: from animal research model to human
  - From one research area to another
  - From one platform to another
- Broaden the reach of your research

### Don't see what you need?

Contact Custom Assay Development Services to:

- Combine analytes from 2 or more multiplex panels
- Develop custom assays on any of our 5 platforms (including RIAs)

# MILLIPLEX<sup>®</sup> assays using Luminex xMAP<sup>®</sup> technology

Rely on the quality we build into each kit to produce results you trust. In addition to the assay specifications listed in the protocol, we evaluate other performance criteria during our validation process: cross-reactivity, dilution linearity, kit stability, and sample behavior (e.g. detectability and stability).

### Each MILLIPLEX<sup>®</sup> MAP kit includes:

- Quality controls (QCs) provided to qualify assay performance
- Comparison of standard (calibrator) and QC lots to a reference lot to ensure lot-to-lot consistency
- Optimized serum matrix to mimic native analyte environment
- Detection antibody cocktails designed to yield consistent analyte profiles within panel

### Intracellular MILLIPLEX<sup>®</sup> MAP panels and kits include:

- Positive and negative control cell lysates provided to qualify assay performance
- Premixed magnetic beads to capture analytes of interest
- Detection antibody cocktails designed to yield consistent analyte profiles within panel



**Primate**  
Cytokine/Chemokine  
Metabolic Hormone  
Pituitary

**Mouse**  
Cytokine/Chemokine  
T Lymphocytes  
Immunoglobulins  
MMPs  
Adipokine/Adipocyte  
Metabolic Hormone  
Pituitary, Thyroid  
Bone  
Cardiovascular  
Angiogenesis  
Neuropeptide  
Kidney Toxicity

**Human**  
Cytokine/Chemokine  
Cytokine Receptors  
Immunoglobulins  
T Lymphocytes  
MMPs, TIMPs  
Complement  
Myokine  
Cardiovascular  
Adipokine/Adipocyte  
Metabolic Hormone  
IGFBPs  
Cancer  
Angiogenesis  
Metastasis  
Liver Proteins  
Pituitary  
Thyroid  
Bone Metabolism  
Skin  
Neurodegenerative  
Neuropeptide  
Kidney/Organ Toxicity

**Porcine**  
Cytokine/Chemokine

**MILLIplex<sup>®</sup>  
Assays**

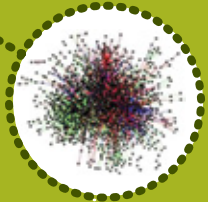
**Equine**  
Cytokine/Chemokine

**Rat**  
Cytokine/Chemokine  
Immunoglobulins  
Adipokine/Adipocyte  
Metabolic Hormone  
Cardiovascular  
Vascular Injury  
Pituitary  
Stress Hormone  
Thyroid  
Bone  
Neuropeptide  
Kidney Toxicity

**Canine**  
Cytokine/Chemokine  
Metabolic Hormone  
Pituitary  
Kidney Toxicity

**Feline**  
Cytokine/Chemokine  
Metabolic Hormone

**Intracellular**  
Y/S/T Phosphorylation  
AKT, MAPK, STAT, SFK,  
NF-κB, TGFβ, RTK, HSP  
Multi-Pathway, TCR  
Apoptosis, DNA Damage/  
Genotoxicity  
OxPhos, Glycolysis  
Oxidative Stress



# Trust your MILLIPLEX<sup>®</sup> MAP data

Trust the quality that we build into each kit. Each panel and kit meets stringent manufacturing criteria to ensure batch-to-batch reproducibility.

## Performance criteria evaluated during the validation process include:

### 1. Antibody specificity

		SFK Multiplexed Capture Beads + Detection Antibodies							
		Src	Fyn	Yes	Lck	Lyn	Fgr	Blk	Hck
Single Recombinant Protein	Src	100%	0.1%	0.3%	0.5%	0.6%	0.2%	0.5%	0.1%
	Fyn	1.0%	100%	2.0%	3.2%	3.5%	1.7%	2.7%	0.5%
	Yes	0.6%	0.4%	100%	1.2%	1.6%	0.7%	1.4%	0.2%
	Lck	0.1%	0.1%	0.2%	100%	0.7%	0.1%	0.3%	0.1%
	Lyn	0.2%	0.1%	0.3%	0.5%	100%	0.3%	0.5%	0.1%
	Fgr	0.6%	0.4%	0.9%	1.7%	2.7%	100%	1.4%	0.4%
	Blk	0.3%	0.2%	0.8%	4.0%	3.1%	0.4%	100%	0.2%
	Hck	0.4%	0.4%	0.7%	5.3%	1.4%	0.4%	1.1%	100%

Table 1. Representative data: Antibody specificity is calculated by MFI testing single standards with multiplexed capture antibody beads and multiplexed detection antibody cocktails.

### 2. Standard curve range, MinDC, precision (intra- & inter-assay), accuracy, dilution linearity, kit stability, and sample behavior (e.g. detectability and stability)

### 3. Comparison of standard (calibrator) and quality control (QC) lots are compared to a reference lot to ensure lot-to-lot consistency

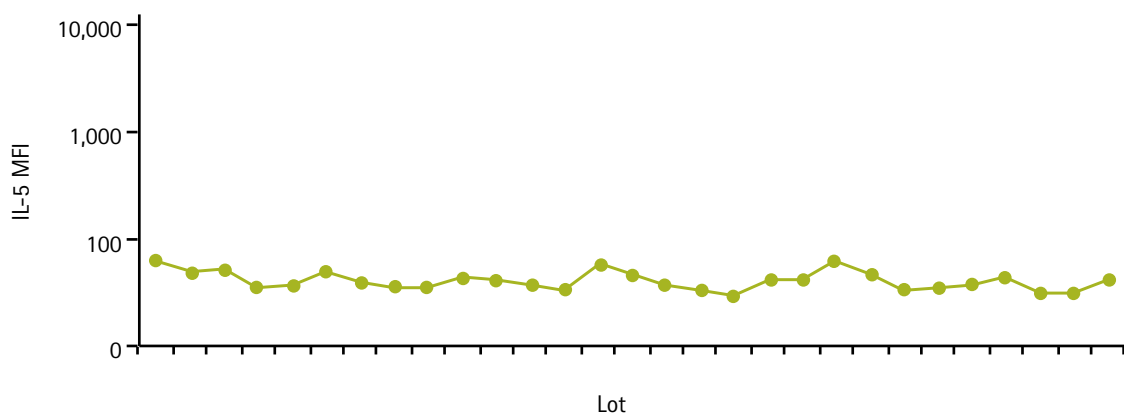


Figure 1. Trend chart shows consistent MFI values for IL-5 standard curve across 29 lots of a MILLIPLEX<sup>®</sup> MAP panel (Cat. No. HCYTOMAG-60K);  $\pm 10\%$  of reference lot.

4. Optimized serum matrix included in each appropriate kit → mimics native analyte environment → results in higher percent recovery for each analyte → improves accuracy of measurement

Average Serum Sample Recovery						
		Sample dilution	IFN $\gamma$	IL-1	TNF $\alpha$	VEGF-A
Standards diluted in assay buffer		Neat	34%	40%	29%	39%
		1:4	49%	63%	52%	51%
		1:20	69%	81%	75%	73%
		Sample dilution	IFN $\gamma$	IL-1	TNF $\alpha$	VEGF-A
Standards diluted in serum matrix		Neat	83%	117%	77%	67%

5. All MILLIPLEX<sup>®</sup> MAP panels include a detection antibody cocktail, designed to yield consistent analyte profiles within panel, regardless of the plex size

Obtain the same reliable quantitation, using the same assay panel, whether running a single-plex or a multiplexed assay.

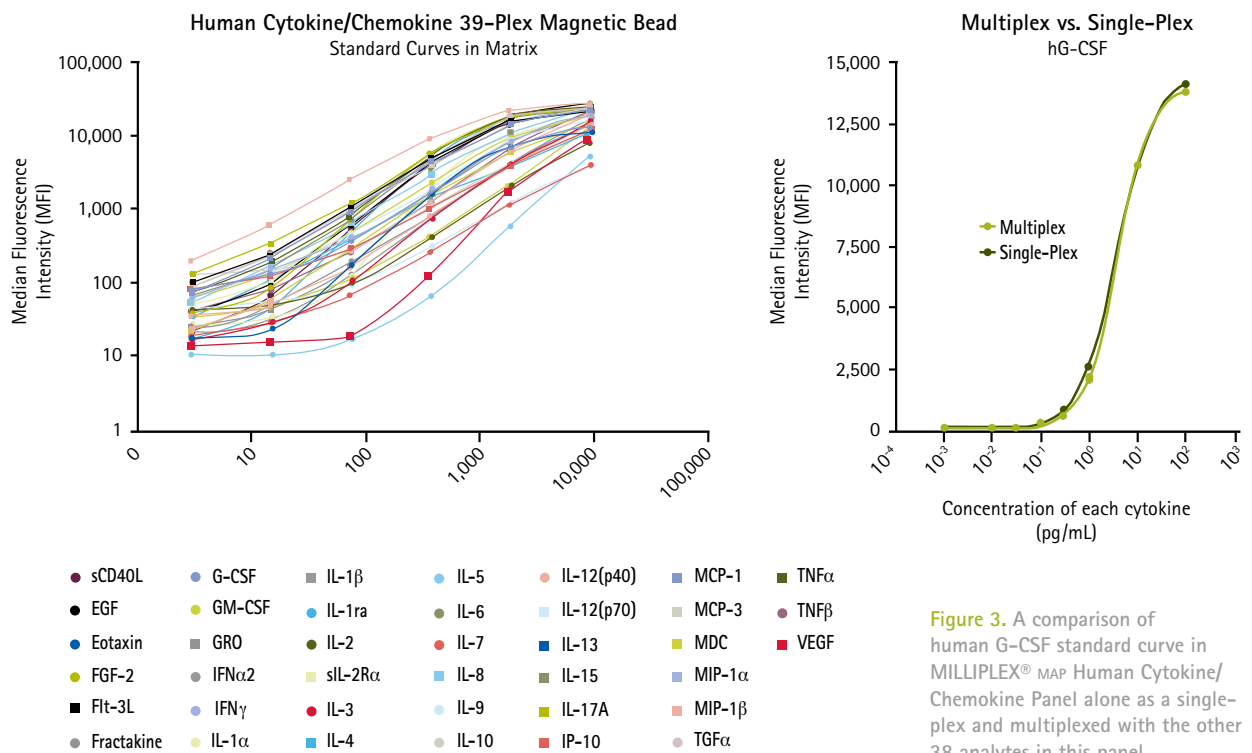


Figure 2. Standard curves of 39 analytes in the Human Cytokine/Chemokine Panel (HCYTOMAG-60K).

Figure 3. A comparison of human G-CSF standard curve in MILLIPLEX<sup>®</sup> MAP Human Cytokine/Chemokine Panel alone as a single-plex and multiplexed with the other 38 analytes in this panel.



# Luminex® Instruments for xMAP® Technology

Complete instrument solutions combined with the largest portfolio of multiplex analytes, MILLIPLEX® Analyst 5.1 software and technical support give you the maximum power of Luminex xMAP® technology for biomarker screening and protein analysis. As a Luminex® partner, Merck Millipore is a preferred distributor of Luminex® instruments, accessories and software.



## MAGPIX® System (Cat. No. 40-072)

- **Low-cost:** Small footprint, low energy system to measure up to 50 analytes with as little as 25 µL sample
- **Powerful:** More than 200 (and growing) MILLIPLEX® MAP magnetic bead kits and single-plex assays—the largest offering of customizable magnetic bead assay panels for the MAGPIX® instrument
- **Easy to use:** Magnetic bead detection using CCD imaging
- **Small and portable:** Saves room on your bench, requiring only 3 ft (91 cm) of space and minimal setup

## Luminex 200™ System (Cat No. 40-012)

- **Flexible:** Run both magnetic and non-magnetic bead assays
- **Multiplex:** Up to 100 analytes per well of a 96-well plate in as little as 25 µL of sample
- **Easy to use:** User-friendly programming
- **Scalable:** Bar code reader included

## FLEXMAP 3D® System (Cat. No. 40-014)

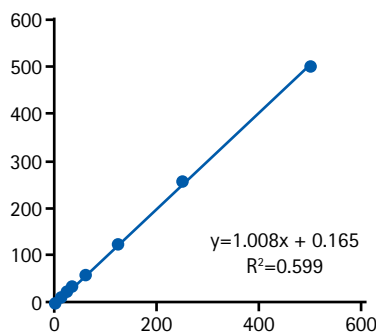
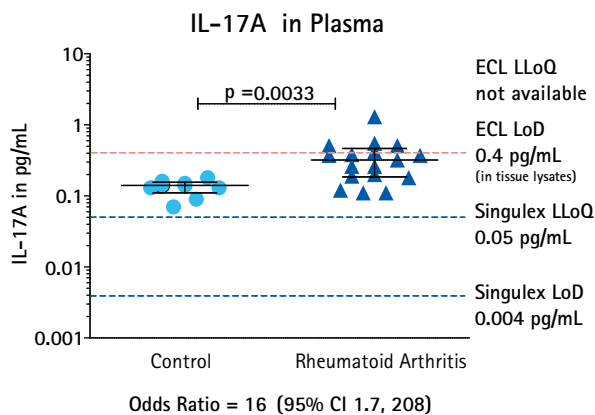
- **Highest multiplexing:** Each bead contains different concentrations of a combination of dyes. Monitoring the three signals enables discrimination of up to 500 different bead sets
- **Ultra-fast:** Quantify 48,000 analytes in under one hour. Dual sample fluidics paths and increased syringe injection speed facilitate faster sample injection
- **96- and 384-well capability:** Greater sample volume flexibility and increased throughput
- **Automation/LIS compatibility:** New xPONENT® 4.0 Software controls the system and offers interfacing options for Laboratory Information Systems (LIS) and automation

# Singulex Erenna<sup>®</sup> platform and Single Molecule Counting (SMC<sup>®</sup>) assays

Singulex Erenna<sup>®</sup> assays, based on single molecule counting (SMC<sup>®</sup>) technology, originally developed by Singulex, Inc., enables ultrasensitive biomarker detection, with lower limits of quantitation in the femtogram/mL level.

## The Singulex Erenna<sup>®</sup> portfolio at a glance:

- Verified immunoassay kits, specific for analytes including cytokines, GLP-1, VEGF, Akt1, and Kim-1
- Plate-based discovery immunoassay kits, featuring low cost, reproducible LLoQ, simple workflow and requiring minimal sample volume
- Prototype immunoassay kits, in multiple areas: neurology, toxicology, metabolism, oncology, inflammation
- Immunoassay development kits for developing your own assays for the Singulex Erenna<sup>®</sup> platform



**Figure 4.** Compared to ECL methods, the Erenna<sup>®</sup> IL-17A assay can more accurately quantify minute differences in plasma biomarker concentrations between age- and gender-matched control and rheumatoid arthritis (RA) patients (top). Data near the lower limit of quantitation (LLoQ) of a Singulex Erenna<sup>®</sup> assay revealed that curve fits exhibited excellent linearity and correlation (bottom).

## Trust your Singulex Erenna<sup>®</sup> data

Verified Singulex Erenna<sup>®</sup> kits are validated using the following criteria:

- **Lower limit of quantitation:**  
Lowest point on standard curve with CV <20% and accuracy within 20% of expected values
- **Inter- and intra-assay precision:**  
Samples run on multiple plates over multiple days  
Spiked and un-spiked samples within 20% across experiments
- **Spike recovery:**  
Minimum of 10 samples spiked with acceptable recovery between 80-120%
- **Dynamic range:**  
Assays target a quantifiable range of 3-4 logs
- **Endogenous range:**  
Minimum of 10 samples from individual donors assessed for ability to quantify baseline biomarker levels

# Erenna<sup>®</sup> Immunoassay System

(Cat. No. 95-0017-00)

The Erenna<sup>®</sup> Immunoassay system, originally developed by Singulex, Inc., accurately, precisely and reliably quantifies low-abundance biomarkers at femtogram/mL concentrations. Ultrasensitive Single Molecule Counting (SMC<sup>®</sup>) technology enables better science by providing better sensitivity.

## Scientists use the Singulex Erenna<sup>®</sup> Immunoassay System when they need:

- Quantification of analyte concentration, especially at ultrasensitive levels
- Data for biomarkers that were previously undetectable or unquantifiable with traditional immunoassay formats
- Unparalleled precision and accuracy, with lower sample volumes and lower analyte concentrations
- A method to rapidly develop their own assays with their proprietary antibodies and targets
- An enthusiastic partner who will work with them to build assays to suit their discovery needs

The Erenna<sup>®</sup> Immunoassay System includes fully integrated benchtop analytical instrumentation with dedicated software for automated data analysis. The instrument captures the sum of all digital events counted. At high concentrations, a proprietary algorithm computes the total sum of all photons recorded.



### Minimum Instrument Performance Specifications

Metric	Specification
Slope	>20 DE/fM*
Background	<100 DE
Limit of detection (LoD)	<1 fM*
Precision	<7% CV†
Dynamic Range	>4 logs

\*Determined from calibrator set: 0-300 fM of 150 kD antibody labeled with fluorophore †DE measurements from 30 fM calibrator, n=20.

# ELISAs

## Measuring single protein biomarkers?



### By choosing our ELISA and RIA kits you'll benefit from:

- 25+ years of experience in metabolic, neuroscience, and cytokine biomarker research
- Stringent quality regulations for development, manufacturing and shipping
- Consistent accuracy and precision, lot after lot
- Key metabolic assays developed to meet the World Health Organization (WHO) standards

### Unlike other kits that leave you on your own to establish reproducibility, our kits provide:

- Comparison of standard (calibrator) and quality control (QC) to a reference lot to ensure lot-to-lot consistency
- Serum matrix for generating standard curves that accurately simulate conditions of native analyte conditions in serum or plasma
- In-assay controls

### Our portfolio of ELISA kits includes over 100 biomarkers, including:

- GLP-1 active
- GLP-1 total
- Rat/Mouse Insulin
- Human Adiponectin
- Human Leptin
- Human GIP

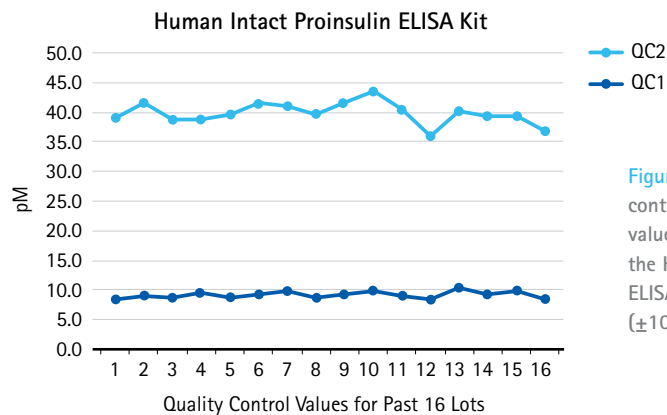


Figure 5. Low and high quality control values show consistent values for the past 16 lots of the Human Intact Proinsulin ELISA kit (Cat. No.EZHPI-15K ( $\pm 10\%$  of reference lot)).

# GyroMark™ HT assays for the Gyrolab® workstation

The Gyrolab® platform delivers high quality immunoassay data over a broad dynamic range, and helps you save time, sample and reagents. This is achieved through precise, automated control of centrifugal and capillary forces to steer liquid flow in proprietary nanoliter-scale microfluidic structures.

## GyroMark™ HT assays provide:

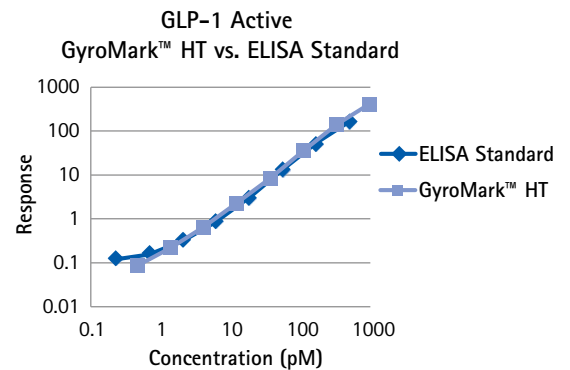
- Accuracy over a four-log dynamic range (compared to two logs for ELISAs)
- Elimination of cross-talk and plate position artifacts
- Simplified sample prep (only 1:2 dilution necessary) with reduced matrix interference

However, developing your own kits can be time-consuming and costly, especially when you need to support a long-term biomarker study through every phase. Building on our 25 years of experience in kit development, we've partnered with Gyros to be the first company to provide high quality, robust and reproducible kits to save you time and money!

## Advantages of using GyroMark™ HT off-the-shelf kits or custom-made assays, compared to developing and validating your own assays for the Gyrolab® workstation:

- Fully qualified, robust and reproducible assays
- High correlation to ELISAs and assays for the Luminex® platform
- Complete your project in days instead of weeks
- Order specific lots of reagents to minimize variation and lot-to-lot drift
- Kits include all the components to run an assay

Our growing GyroMark™ HT portfolio includes assays for GLP-1 Active, KIM-1, insulin, IL-6, TNF $\alpha$ , and more.



**Figure 6.** High correlation to ELISA data. Excellent overlap of GLP-1 Active standards between the GyroMark™ HT Multi-Species GLP-1 Active Assay (Cat. No. GYGLP1A-35K) for the Gyrolab® xP Workstation and Merck Millipore's Multi-Species GLP-1 Active ELISA assay (Cat. No. EGLP-35K). Sample volume was 1,000 nL and samples were diluted 1:2.

Made to Order

# Full-service, Custom-built Assays

We let YOUR science drive biomarker assay development.

## Select your assay platform:

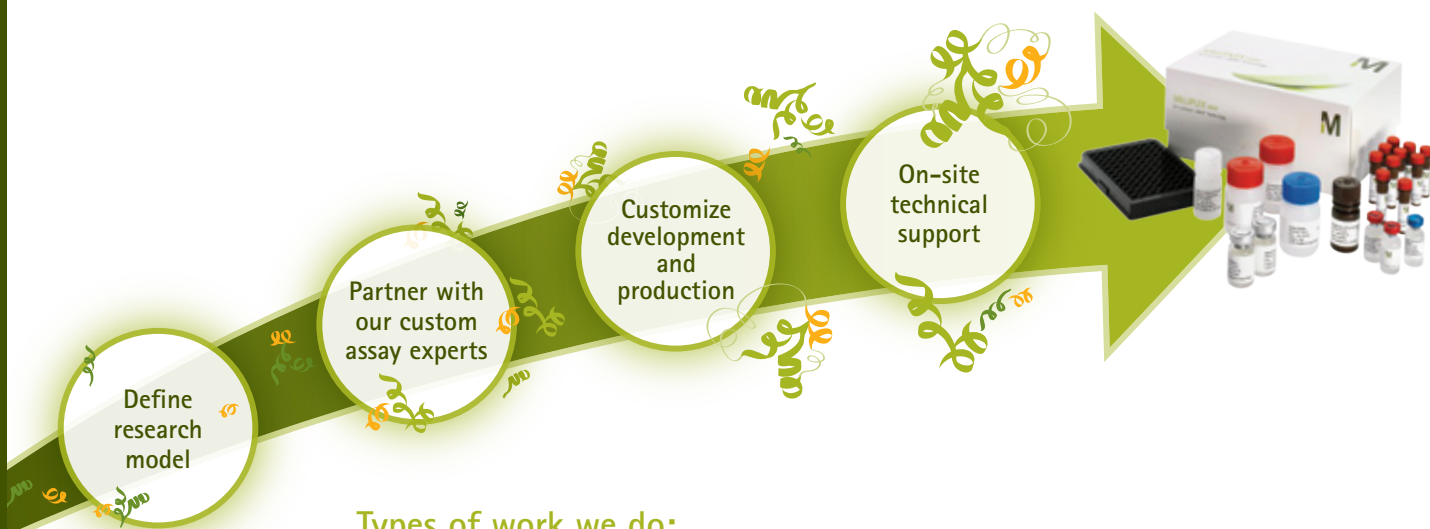
- Luminex® systems
- Singulex Erenna® platform
- ELISAs/RIAs
- Gyrolab® workstation

## We deliver:

- Leading partner for assay development
- More than a century of combined immunoassay development experience
- Full analytical validation
- Project-specific quality control
- High-throughput screening

## Get the degree of customization you need:

- Combine existing assays from our broadest collection of analytes available
- Receive complete protocols, analytical validation and all the reagents you need in a single kit
- Got an antibody? We can use it to develop an immunoassay on your platform of choice; for example, we can conjugate it to xMAP® beads and validate for your Luminex® system
- No antibody yet? We'll develop one for you, carefully choosing immunogen and appropriate screening and validation



## Types of work we do:

- Global, standardized bioanalysis for multi-site studies
- Customized quality control and assay validation to meet your project needs
- Add assays for new biomarkers to existing multiplex assay panels
- Design and develop bioanalytical studies to support your translational or investigational research
- ... and more!

Consult with our custom assay team to learn more about how our extensive assay design experience can be customized for your project needs. Visit: [www.merckmillipore.com/customassay](http://www.merckmillipore.com/customassay)

## To place an order or receive technical assistance

In Europe, please call Customer Service:

France: 0825 045 645

Germany: 069 86798021

Italy: 848 845 645

Spain: 901 516 645 Option 1

Switzerland: 0848 645 645

United Kingdom: 0870 900 4645

For other countries across Europe,  
please call: +44 (0) 115 943 0840

Or visit: [www.merckmillipore.com/offices](http://www.merckmillipore.com/offices)

For Technical Service visit:

[www.merckmillipore.com/techservice](http://www.merckmillipore.com/techservice)



[www.merckmillipore.com/biomarkerassays](http://www.merckmillipore.com/biomarkerassays)

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