

Single-molecule quantification of protein biomarkers with the Erenna® Immunoassay System.

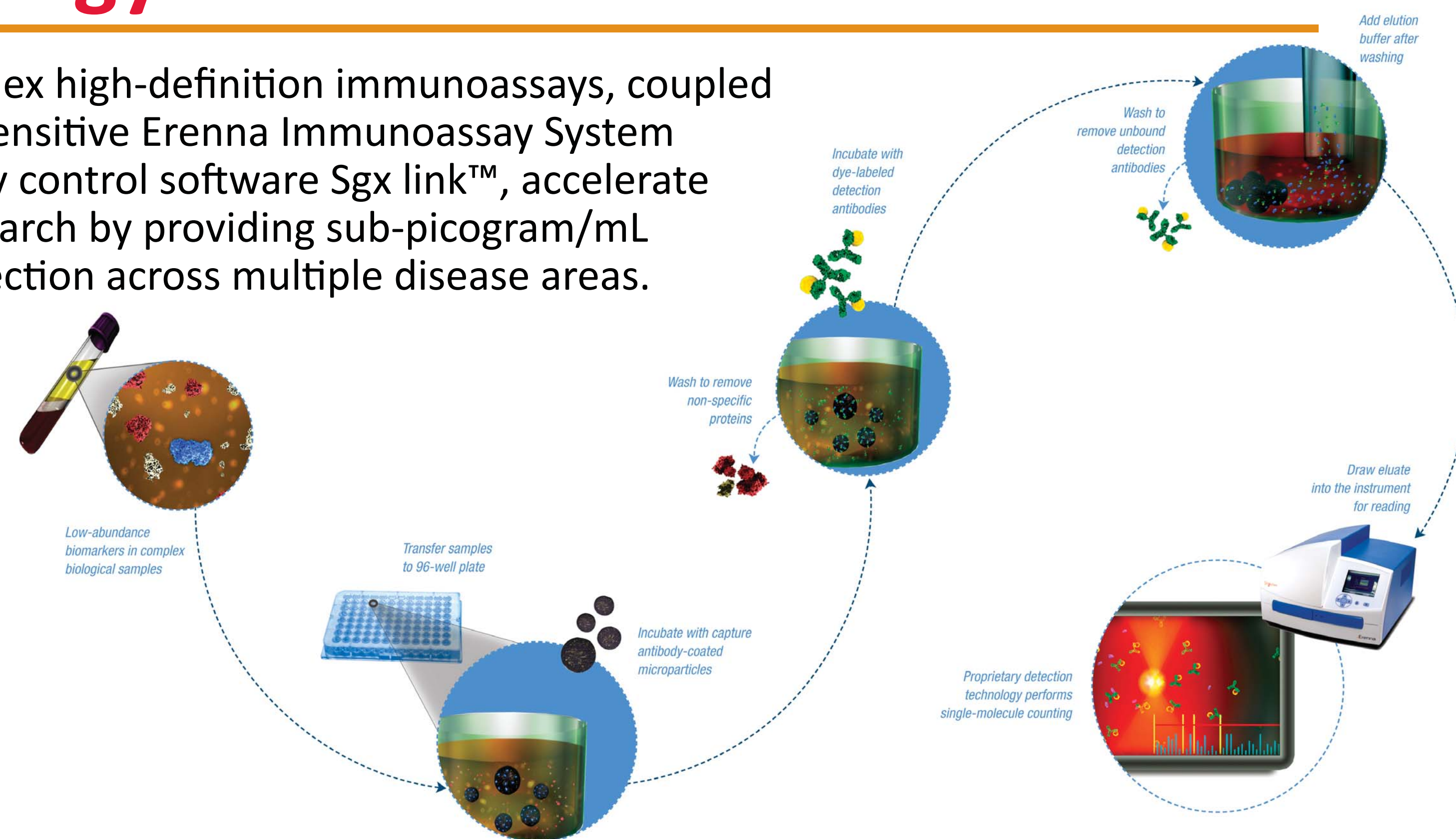
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Abstract & Introduction

Singulex provides high-definition immunoassay products and services for biomarker development to accelerate pharmaceutical, clinical and life science research. Our patented Erenna® Immunoassay System is an innovative digital technology that measures single molecules at sub-picogram per mL concentrations. Using this platform, we have developed a broad menu of ultrasensitive cytokine immunoassays which provide high-definition monitoring of baseline biomarker concentrations in healthy individuals. These assays can be utilized to assess the role of inflammatory cytokines in multiple disease states. Here we present several case studies applying the Erenna Immunoassay System with respect to improving the clinical utility of protein biomarkers. We also demonstrate application of the Erenna Immunoassay System towards pre-clinical and clinical biomarker qualification and verification across multiple disease and therapeutic areas. Taken together, we demonstrate that single-molecule technology can be used in programs that advance protein biomarkers as surrogate markers of disease, efficacy markers for novel therapies, or companion diagnostic markers. By incorporating advanced single molecule technology and taking a pathway-forward approach, pharmaceutical and life science researchers can avoid risk factors that contribute to attrition and therefore accelerate pharmaceutical development by reducing drug development timelines.

Technology

Figure 1. Singulex high-definition immunoassays, coupled with the ultrasensitive Erenna Immunoassay System and proprietary control software Sgx link™, accelerate biomarker research by providing sub-picogram/mL biomarker detection across multiple disease areas.



Analytical Performance

Table 1. Singulex assays offer greater sensitivity over competitive methods, allowing baseline biomarker levels to be accurately measured in healthy volunteers (HV).

Assays	Singulex			R&D Systems			MesoScale Discovery			
	Analyte	Sample Type(s)	LoD (pg/mL)	LLOQ (pg/mL)	[Analyte] in HV Plasma (pg/mL)	LoD* (pg/mL)	LLOQ* (pg/mL)	[Analyte] in HV Plasma (pg/mL)	LoD† (pg/mL)	[Analyte] in HV Plasma (pg/mL)
IFN-γ	Plasma	0.14	0.2	2.7-6.7	8	15.6	<15.6	NA	NA	NA
IL-1β	Plasma	0.01	0.04	0.1-0.2	0.06	0.125	ND-0.4 (25% det)	2.6	ND	ND
IL-6	Plasma	0.01	0.1	0.6-3	0.1	0.1	0.4-8.8	1.6	ND-4.7 (20% det)	NA
IL-17A	Plasma/Serum	0.01	0.05	0.1-0.6	15	31	<3.9	0.2	NA	NA
IL-17F	Plasma/Serum	0.60	1.6	5.1-12	200	200	<200	NA	NA	NA
hTNFα	Plasma	0.01	0.02	0.7-3	0.12	0.5	ND-4.2 (97% det)	0.7	ND-2.2 (20% det)	NA
hVEGF	Plasma	0.04	0.15	5.1-18	<5	15.6	ND-115 (24% det)	NA	NA	NA

*R&D LoD: mean of "minimum detectable dose" background + 2*standard deviation/slope.
 †R&D LLOQ: lowest calibrator concentration on the standard curve.
 ‡MesoScale Discovery LoD: "detection limit" background + 2.5 * standard deviation.

Case Study: High Profile Clinical Studies

Challenge: Early-stage multi-panel biomarker screening lacks a robust technology to produce conclusive data and support early rule-in/rule-out decisions in clinical studies.
Singulex Solution: Singulex provides high-definition immunoassays that can be customized on an ad-hoc basis to power large-scale multi-marker clinical studies. The sensitivity provided by Singulex allows endogenous biomarker concentrations to be quantified in as little as 1 μL of sample volume, further conserving archival samples.

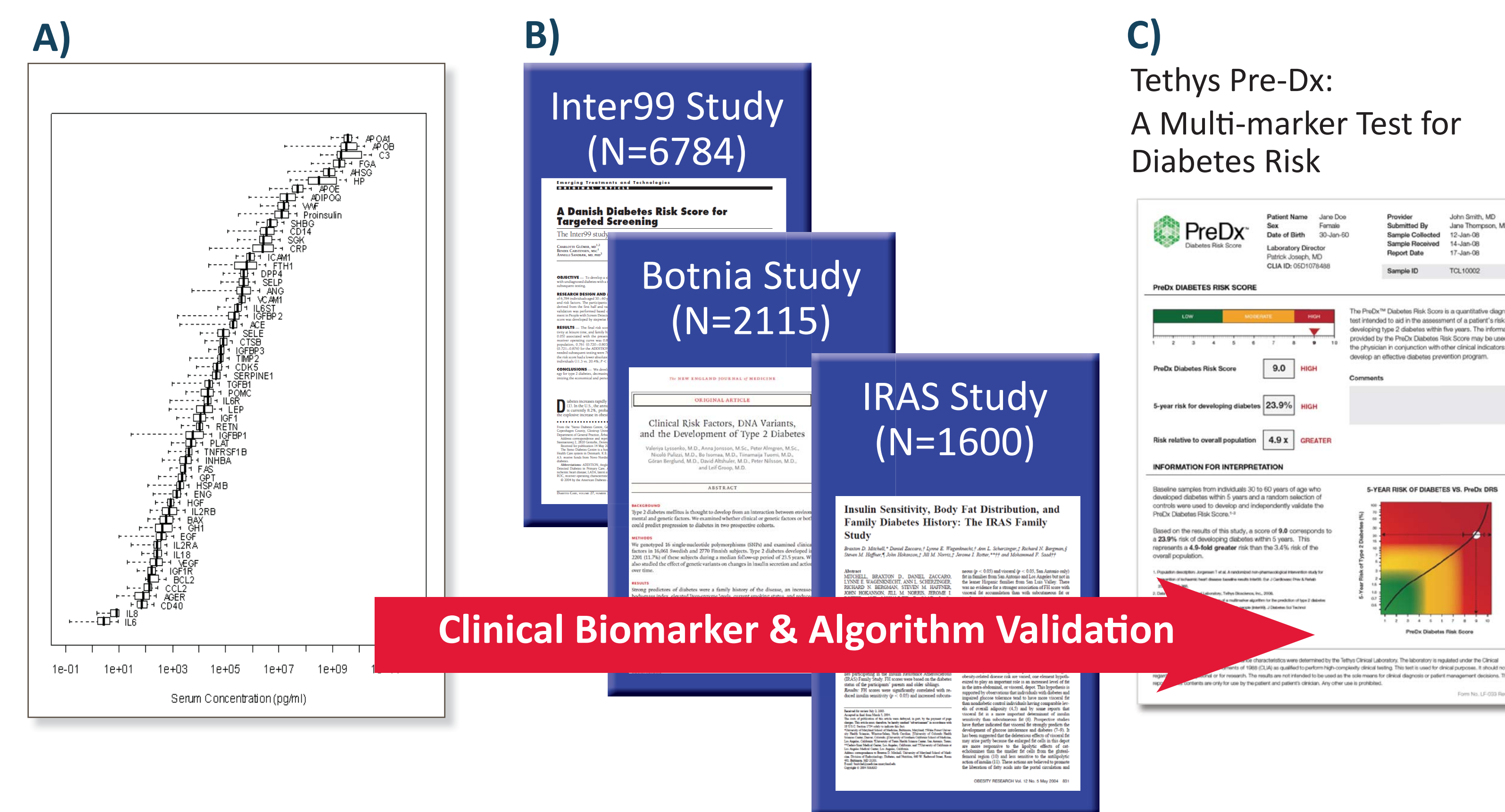


Figure 2. A) A panel of 58 biomarkers were screened using Singulex technology, using as little as 1 μL of sample volume per test. B) Biomarkers with disease relevance were further validated in population-based studies. C) Scoring algorithms involving biomarkers with clinical utility were validated as a tool for assessing disease risk.

Case Study: RA Inflammation

Challenge: Inadequate detection method for quantifying low-abundance biomarkers implicated for inflammatory diseases, in particular rheumatoid arthritis.
Singulex Solution: Singulex provides high-resolution monitoring of baseline IL-17A concentrations that are present at low levels, allowing for a clear differentiation of the disease state from the healthy population.

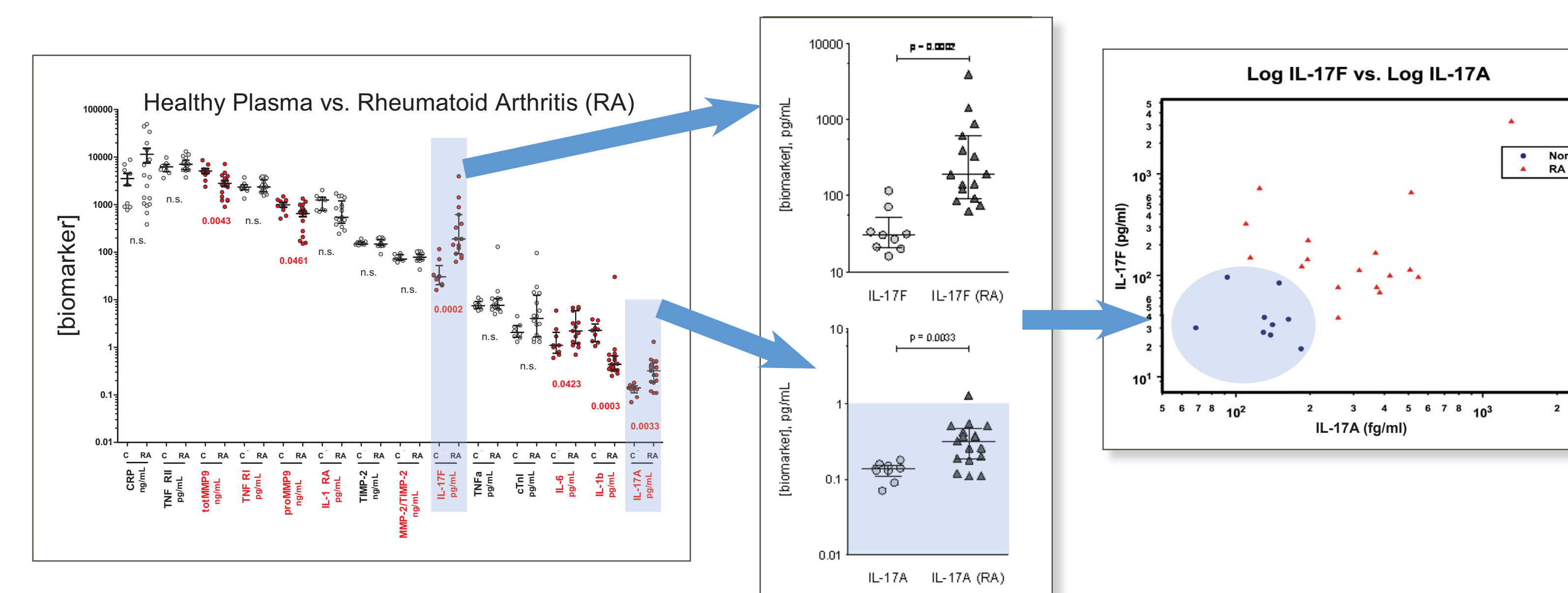


Figure 3. A multi-marker analysis of rheumatoid arthritis patients (RA) vs healthy volunteers (HV) was conducted using the Singulex technology. The study identified markers with significant concentration differences between healthy and disease populations. In particular, Singulex provides the sensitivity and quantifiability needed to accurately measure baseline IL-17A concentrations in HV, as well as minute elevations in RA. In conjunction with data from IL-17F, this dual marker differentiation powered by Singulex allows for 100% specificity for RA patients in this study.

Case Study: Kinase Oncology

Challenge: Insufficient sensitivity with commercially available assays to measure MAPK and PI3 kinases directly in cell lysates.
Singulex Solution: Singulex provides iso-form and phosphorylation-specific kinase assays to elucidate the clinical relevance and potential diagnostic utility of kinases with a high degree of specificity.

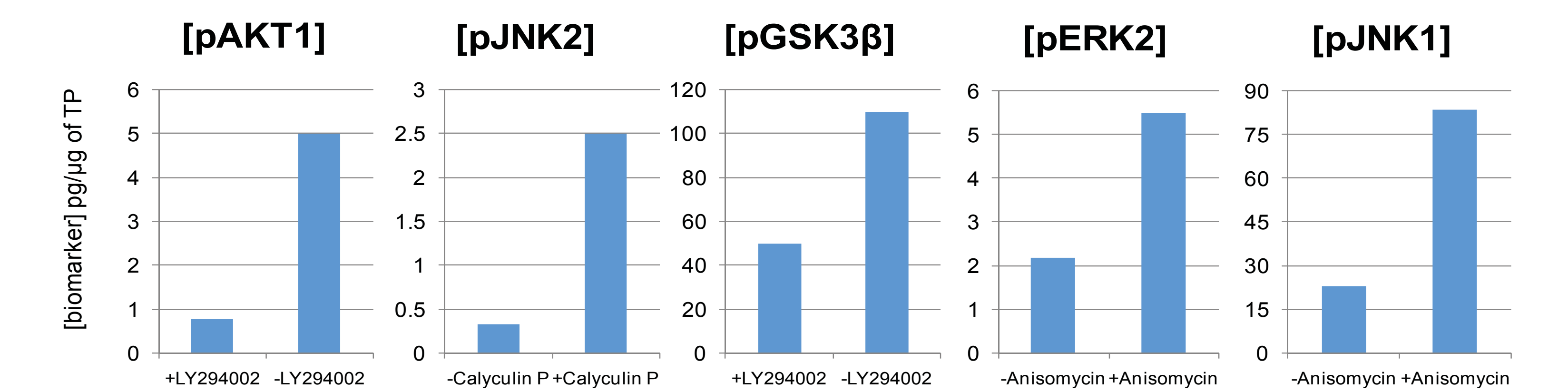


Figure 4. Phospho-specificity exhibited by the Singulex phospho-kinase assays, as tested in Jurkat cells treated with selective inhibitors of the MAPK (LY294002) pathway, or with broad activators of intracellular phosphorylation (Anisomycin or Calyculin Pervanadate).

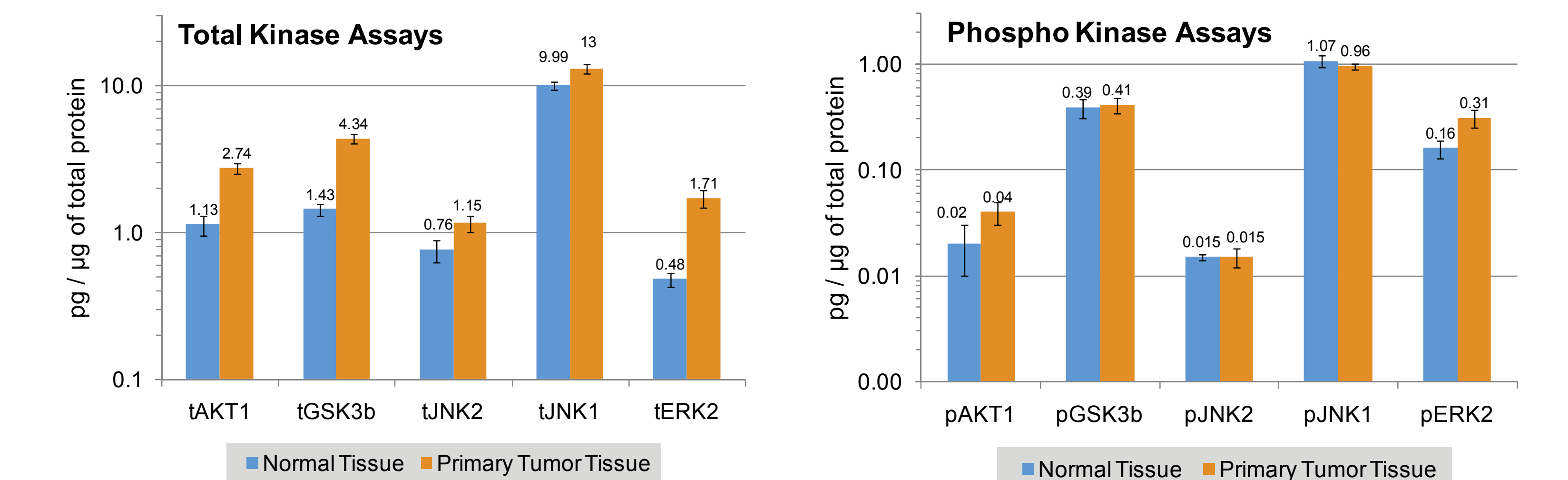


Figure 5. Mean biomarker concentrations of matched normal (■) and primary (■) breast cancer tissue lysates from a single individual using the full Singulex Kinase Assay Panel. Significant elevations (P ≥ 0.05) of total, but not phosphorylated, kinases were observed in primary tumors compared to surrounding adjacent normal tissue.

Conclusion:

- The patented single-molecule counting technology provided by Singulex offers superior sensitivity over competitive methods, enabling development and clinical validation of disease-relevant biomarkers.
- With lower limits of quantification, only a minute sample volume is required for the determination of biomarker concentration at baseline. This allows precious sample to be conserved from high-profile clinical study specimens, and multi-panel studies to be performed.
- Due to the high-resolution power of Singulex technology, researchers can make new discoveries in disease areas such as inflammation and oncology, even using small study populations.



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