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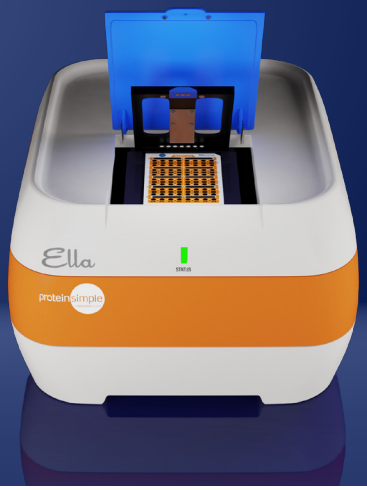
APPLICATION NOTE

SIMPLE PLEX:

THE HANDS-FREE, LOW-VOLUME, MICROFLUIDIC ELISA ALTERNATIVE



INTRODUCTION

Standard ELISA techniques for detecting protein biomarkers in serum or other biological samples leave you with sensitivity that doesn't quite cut it, analyte cross-reactivity, and reproducibility that's ok at best. They're also pretty low throughput and need a lot of hands-on time. And each manual step introduces variability, so the only way to rule out human error is to run samples in duplicate. All of these drawbacks have prevented adoption of ELISA techniques when testing complex, multivariate diseases like sepsis[1], rheumatoid arthritis[2], cancer[3], neurodegenerative diseases[4] and traumatic brain injury.[5]

Ella automates all the steps of a Simple Plex™ assay and gets rid of many traditional ELISA challenges. So you'll get more precise data without all the hassle in just one hour. It all happens on disposable Simple Plex cartridges which currently come in a variety of single-analyte and multi-analyte configurations. Three mini ELISA replicates happen in multiple independent microfluidic channels, so there's no cross-reactivity from antibody pairs for other assays, and you get triplicate data for each sample. So you'll improve your sensitivity and be able to detect multiple analytes in a single sample – all with as little as 25 µL per sample.

In this application note, we'll explain how it all works and show you how Simple Plex assays give you better sensitivity and reproducibility than ELISA for protein biomarker detection in serum.

WHY CHOOSE SIMPLE PLEX OVER OTHER PLATE-BASED ELISAS?

Simple Plex assays beat other ELISAs hands-down in both quality of assay results and workflow (**TABLE 1**). Not only is it a low volume assay that you can set up in just a few minutes, you'll also get rid of repetitive steps like manual washes or complicated reagent additions because Ella automates everything on the cartridge. And to make things even better, each cartridge assay is certified using R&D Systems™ reagents and has its own factory-generated standard curves.

You'll have high-quality results in an hour, unlike the many hours you'd spend in preparation waiting for results with other ELISA formats.[11, 12] Simple Plex assays also use fluorescence detection, which improves your sensitivity by 10X and boosts your dynamic range up to 5 logs. Multiplexing results are a whole lot better too. With the multi-analyte cartridges, there's no possibility of getting the antibody cross reactivity that's common with multiplexed ELISAs[6,7] because your sample is split across four distinct channels. You'll also get the same specificity you do with your current ELISA – just with better reproducibility and the ability to detect lower analyte levels than you can now with current technologies.[8,9,10]

IT'S ALL ABOUT THE GLASS NANO REACTORS

Simple Plex assays take place in glass nano reactors (GNRs) – they're the solid-phase support for the capture antibodies used for the sandwich immunoassay. GNRs were designed to make sure your antibodies only immobilize on the GNR's internal surface. Plus, its highly uniform surface results in low intrinsic fluorescence, yet another reason why your assay sensitivity will go up a notch or two.

	PLATE-BASED ELISA	SIMPLE PLEX
QUALITY OF ASSAY		
Validated R&D Systems Reagents	No	Yes
Multi-analyte	No	Yes
Sample Volume Required[11,12]	50–200 µL	25 µL
Typical Reproducibility[8,9]	15%	7%
Sensitivity[8,9,10]	>10 pg/mL	≤1 pg/mL
Cross-Reactivity Risk[6,7]	Yes	No
Dynamic Range	1–3 logs	4–5 logs
Standard Curves Included	No	Yes: factory-generated curves
EASE OF OPERATION[11,12]		
Assay Setup Time	80–120 minutes	10–15 minutes
Time to Run Assay	4 hours	~ 1 hour
Number of Wash Steps	Many	None
Results Per Sample Well	Single data point	Triplicate data points
Sample Throughput/Day	40–120	72- 420 (Up to 6 cartridges)
Instrument Maintenance	If automated, cleaning & calibration	No user maintenance required

TABLE 1. Simple Plex assays do better in all areas of assay preparation, system operation and data quality than standard plate-based ELISAs.

LOSE THE CROSS-REACTIVITY

The Simple Plex cartridge has either 16, 32, or 72 microfluidic circuits depending on the format you're using (**FIGURE 1**). These circuits have all the pneumatically actuated pumps and valves needed to control liquid flow from the various sample inlets and reagent reservoirs. The circuits also use discrete microfluidic channels that eliminate any chance of analyte cross-reactivity, and each has three GNRs for triplicate analyte detection. So why is having three GNRs per microfluidic channel a good thing? It means you'll automatically get triplicate data for each of your samples.

EASY ASSAY SETUP

Simple Plex assay setup is easy. First, centrifuge your samples at 20,000 x g in a microfuge for 10 minutes to isolate the supernatant samples. Then scan the cartridge barcode and prepare the assay by diluting your samples in sample diluent. Transfer your diluted samples directly into the cartridge's sample inlets, and add 1 mL of wash buffer into each wash buffer inlet. Next, dilute the appropriate controls from R&D Systems for each analyte at high and low concentrations based on the instructions in your product insert, and add 50 µL of each to two sample inlets. Then just put the cartridge in Ella, hit Start and she'll do all the rest (**FIGURE 2**).

HANDS-OFF FROM START TO FINISH

Because Simple Plex cartridges use microfluidic circuits to run all steps of a sandwich ELISA assay, no manual wash steps are needed. Ella automates every step (**FIGURE 3**), from sample incubation to analyte detection.

Analyte-specific calibration curves are assigned to every cartridge lot during manufacture, so you don't have to create your own. When you scan the cartridge barcode, Ella knows which assays you're running and Simple Plex Runner software also pulls up the associated calibration curve(s). When the run's done, Simple Plex Explorer software automatically back-fits concentration results from samples and controls to the analyte-specific calibration curve to give you the calculated mean recovery and CVs. And if you need to, you can also export your data as a CSV file into Microsoft® Excel® for further analysis.

SENSITIVITY AND STANDARD CURVES THAT BEAT ELISA

We evaluated specific capture and detect antibody pairs for the cytokines IL-1β, IL-5, IL-10 and IL-12 in spiked serum samples for LOD, LLOQ, and ULOQ (**TABLE 2**). Our standard curves for each biomarker resulted in pg/mL detection limits, a 3–5 log dynamic range and CVs of 10% or less (**FIGURE 4**)! Natural samples also correlated with the Quantikine™ ELISA standard curve for each analyte, yielding an R^2 value of greater than 0.9 for each analyte.

SPOT-ON SELECTIVITY

Selectivity and recovery of each biomarker was also assessed in serum samples at concentrations near LLOQ, below ULOQ, and midway between the two. Recovery was defined as backfit concentration of the spiked sample subtracted by the endogenous level, divided by the sum of the amount measured in the spiked control sample.[13] Assay specifications required a mean biomarker intra-assay recovery of 80–120% with ≤25% CVs and a 70–130% inter-assay recovery with ≤25% CVs for a minimum 83% of the samples tested.[14]

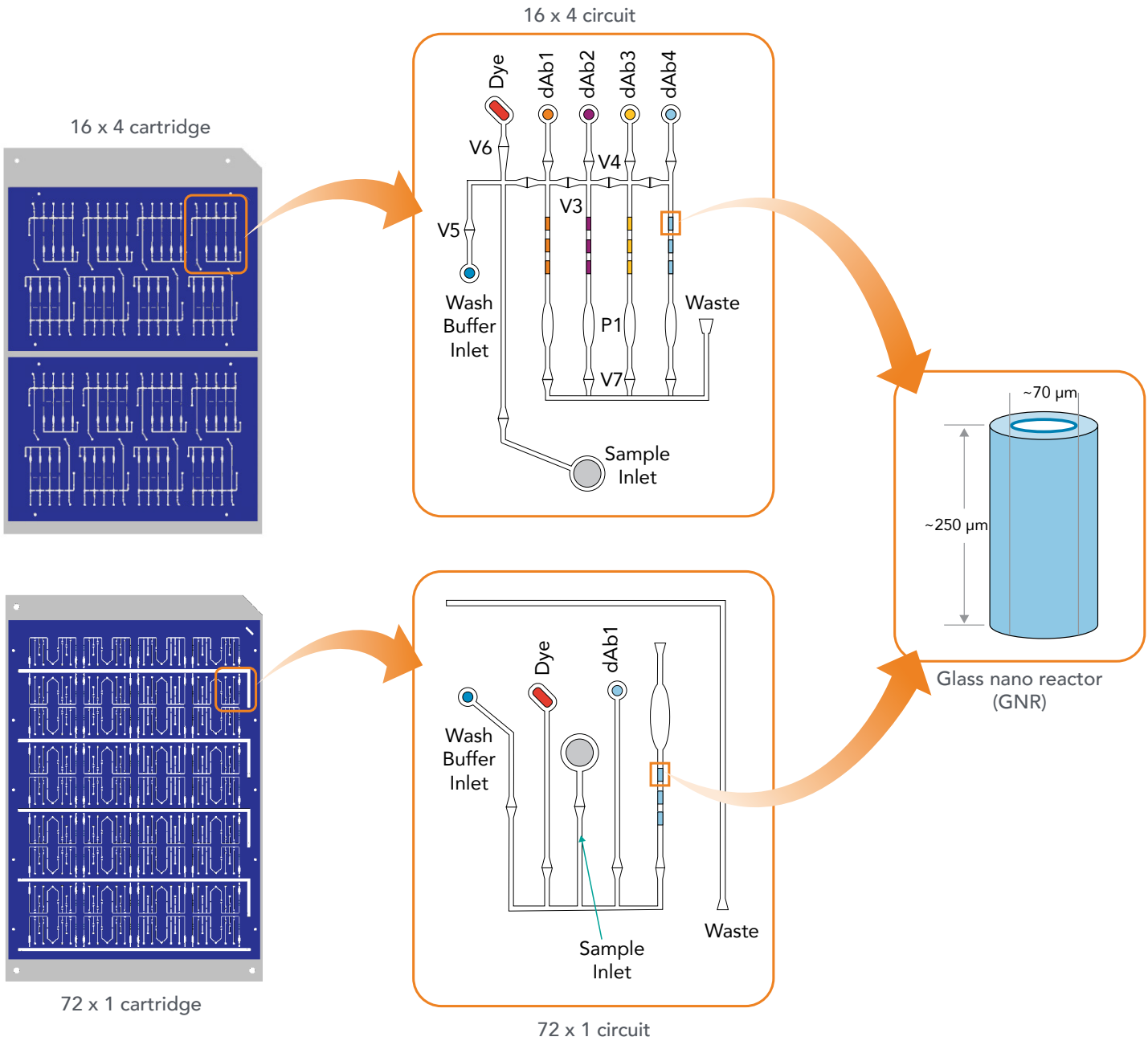


FIGURE 1. Bottom view of 16 x 4 (top, left) and 72 x 1 (bottom, left) cartridges. These cartridge examples contain either 16 microfluidic circuits that can analyze up to four analytes (top, middle) or 72 microfluidic circuits that can analyze one analyte (bottom, middle). Each circuit contains discrete channels with three GNRs that are 250 mm x 70 mm ID (right).

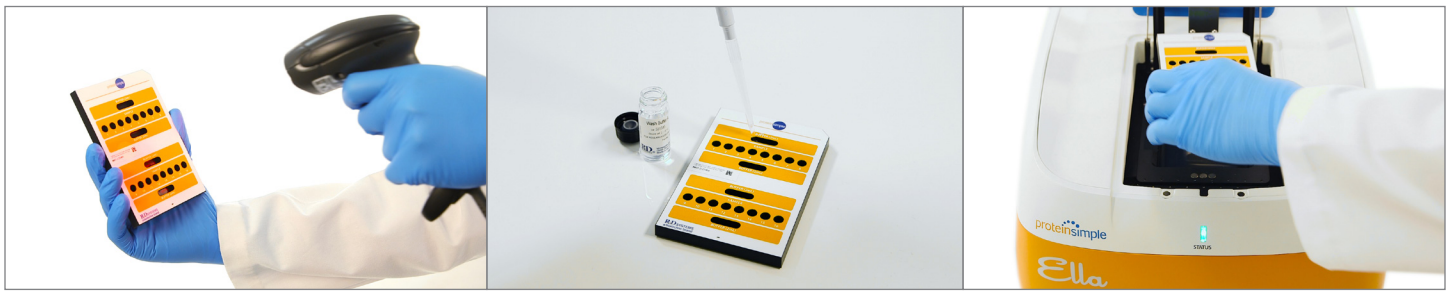


FIGURE 2. Assay setup for a 16 x 4 or a 72 x 1 cartridge.

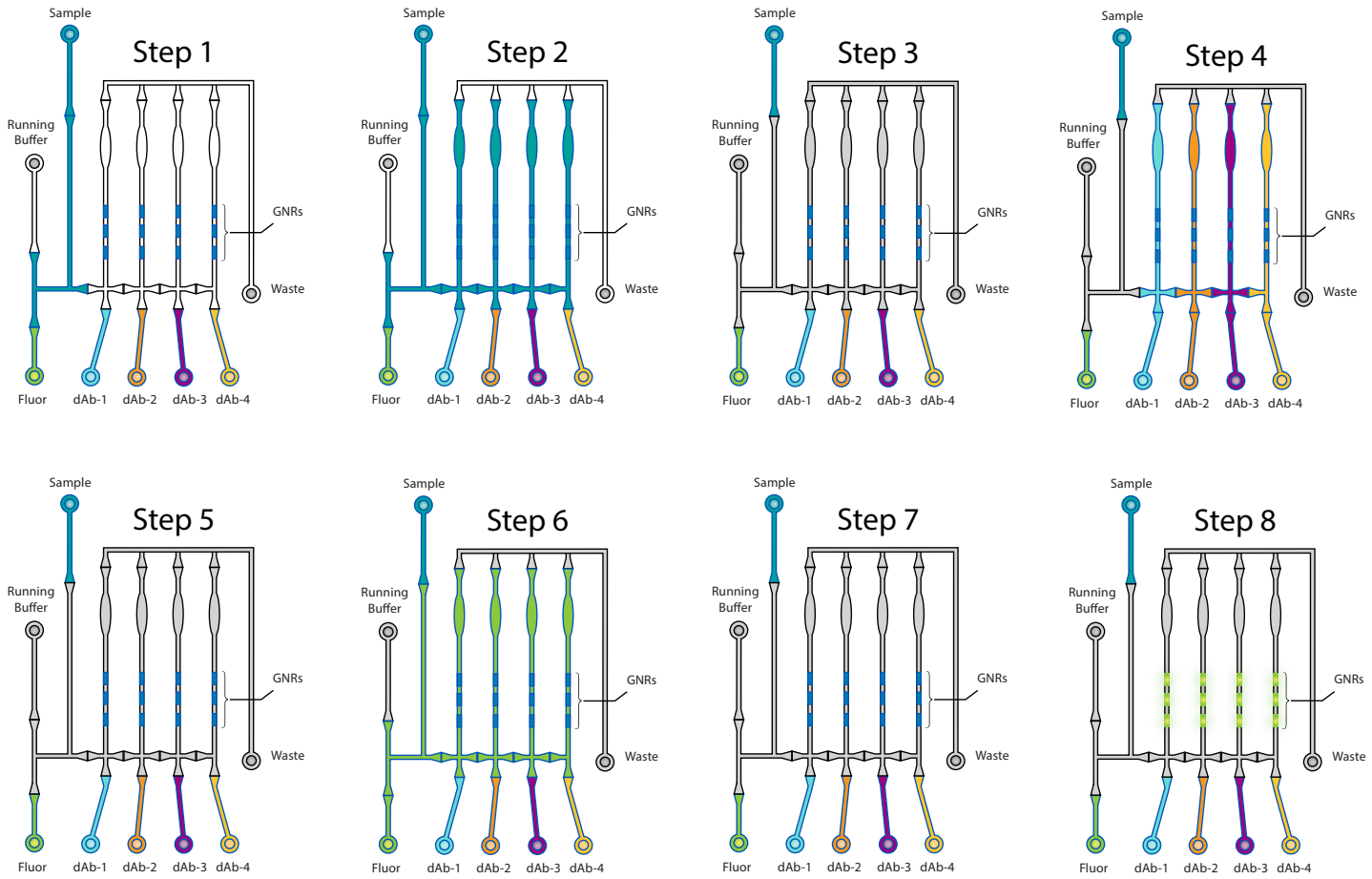


FIGURE 3. The Simple Plex assay work flow, shown for a multi-analyte. Step 1: Prime the system with sample. Step 2: Sample is split evenly between the four channels containing analyte-specific GNRs. For the 72 x 1 circuit, the sample moves into the channel containing the GNRs. Step 3: Buffer washes the circuit after sample incubation. Step 4: Pump analyte-specific dAbs into their respective channels to bind analyte captured on the GNRs. Step 5: Remove unbound dAbs with wash buffer. Step 6: Detect fluor binds bound dAbs. Step 7: Remove residual detect fluor with wash buffer. Step 8: Excite detect fluors with a 631 nm laser and read signal with a CCD camera.

ANALYTE	LOD pg/mL	LLOQ pg/mL	ULOQ pg/mL	MEAN % RECOVERY	INTRA-ASSAY VARIATION (% CV) – LOW QC	INTRA-ASSAY VARIATION (%CV) – HIGH QC	INTER-ASSAY VARIATION (%CV) – LOW QC	INTER-ASSAY VARIATION (%CV) – HIGH QC
IL-1β	0.064	0.21	840	110	4.9	3.7	4.0	5.7
IL-5	0.02	0.069	3120	99	7.5	5.5	10.1	8.3
IL-10	0.14	0.46	5530	94	4.6	6.0	7.1	7.1
IL-12	0.39	0.460	2570	81	3.4	5.4	5.9	8.6

TABLE 1. Simple Plex assays result in low analyte detection limits and assay variation at or below 10% CVs. The LOD was calculated by adding 3 SD to the mean background signal determined from multiple runs. Mean % recovery was determined in human serum spiked with three different concentrations within the assay range.

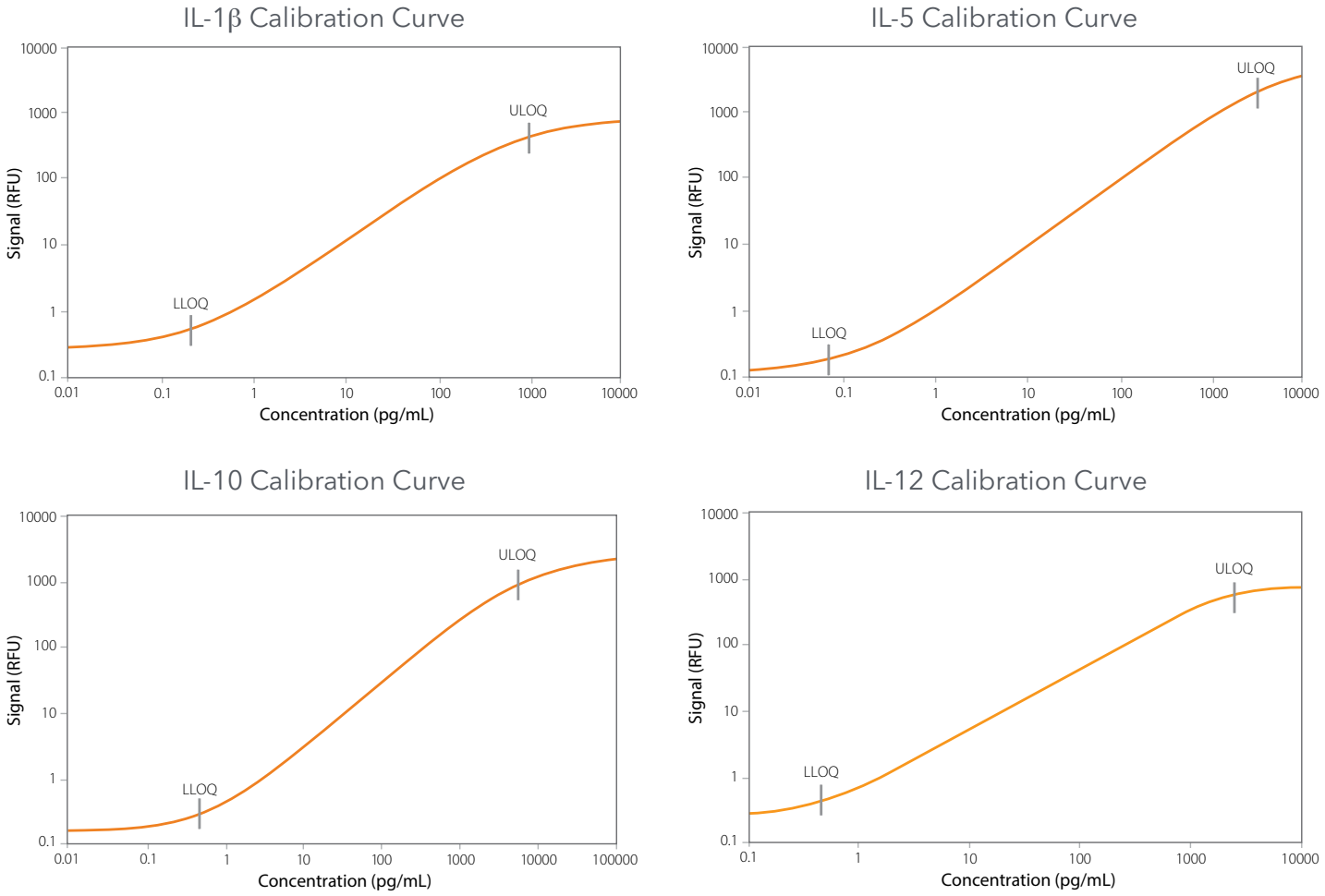
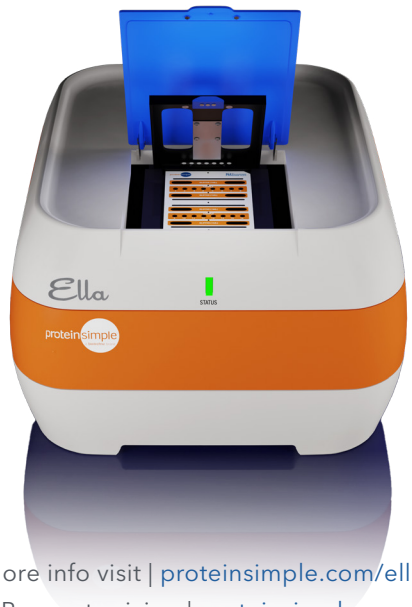


FIGURE 4. Calibration curves for the individual biomarkers run using the Simple Plex assay. The factory-generated calibration curves shown were compiled by averaging five replicates of each calibrator from multiple runs. The 5PL curve fit shows the calibrator concentration (pg/mL) as a function of signal intensity (relative fluorescent units, RFU).

CONCLUSION

Simple Plex assays eliminate a lot of the limitations that come with measuring protein biomarkers using plate-based, single-and multiplex-analyte ELISAs. Assay setup takes less than 15 minutes and once you hit Ella's start button, she automates the whole process for you. So there's no manual wash steps that up both your hands-on time and your assay variability. Depending on the cartridge you pick, you can analyze a single analyte, or up to 8 analytes simultaneously. Simple Plex assays are also low-volume, so you only need 25 µL of sample. That's 2–4X less than ELISA!

Because these fluorescent immunoassays happen in individual microfluidic channels, reaction kinetics are fast with zero cross reactivity. Now you'll get up to 10X more sensitivity and a dynamic range of 3–5 logs in the process. And all your standard curves are built in! Our experiments resulted in LODs under 1 pg/mL with CVs of 10% or less for IL-1β, IL-5, IL-10 and IL-12 in serum samples.



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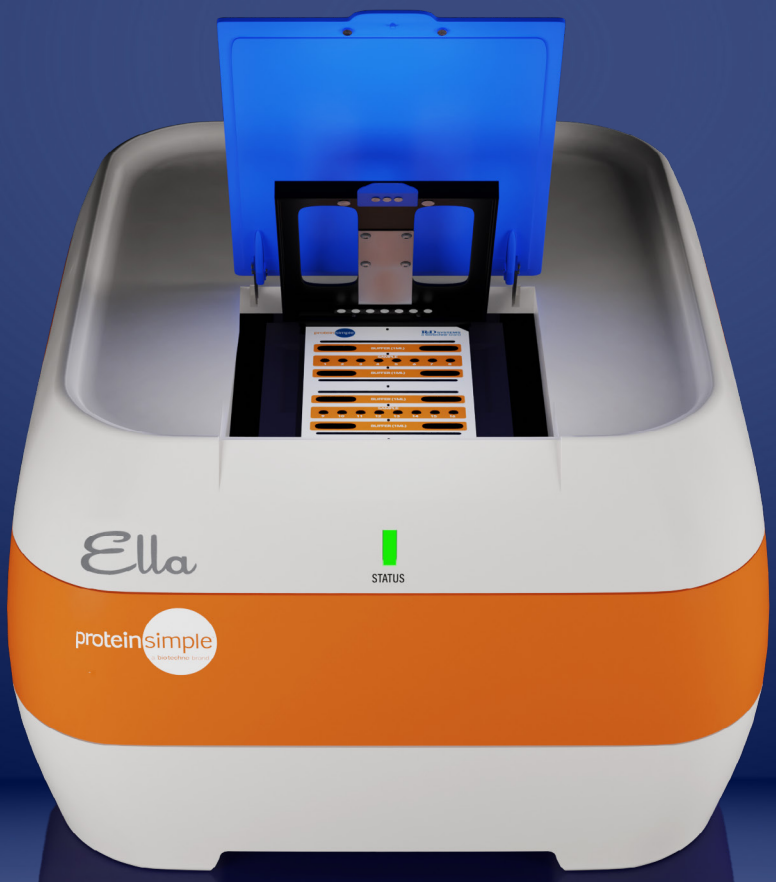
ELLA

YOUR NEXT GENERATION ELISA



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