Accelerating high-throughput screening with FirePlex®-HT:

An automatable, multiplex immunoassay using FirePlex® Particle Technology

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In patient samples and animal models, molecular biomarkers are used as indicators of normal and pathogenic processes. For drug discovery and screening pipelines, molecular biomarkers are used to assess mode of action, efficacy, and toxicity of lead compounds. Here we introduce the FirePlex®-HT multiplex immunoassays, which enable rapid and sensitive quantitation of up to 10 protein biomarkers from small sample inputs. The two-step workflow and no-wash, 384-well plate assay format limit hands-on time and are amenable to automation, thus making FirePlex®-HT ideally suited for high-throughput screening studies.

Introduction

To address the need for rapid and sensitive quantitation of biomarkers from biological samples, we have developed the FirePlex® Technology Platform. Utilizing patented FirePlex hydrogel particles and a three-region encoding design, FirePlex Immunoassays allow for true, in-well multiplexing, providing flexible and customizable quantification of analytes.

Our high-throughput FirePlex (FirePlex®-HT) Immunoassays quantify up to 10 protein analytes per well from low sample inputs, in 384-well plate format. The two-step workflow and no-wash assay format limit hands-on time, and are amenable to automation. Assay readout is conducted on high-content imagers with data analysis using the FirePlex® Analysis Workbench software, bypassing the need for dedicated instrumentation and expensive software licenses.

FirePlex particle overview

At the heart of the FirePlex-HT assays is the innovative FirePlex particle technology (patented porous bio-inert hydrogel) which enables high-performance multiplexing capabilities and easy readout on high-content imagers.

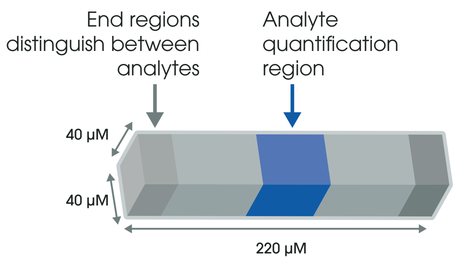


Figure 1. FirePlex particles provide optimal thermodynamics, detection directly from biofluids and is decoded by our integrated analysis software

Assay specifications

Workflow format	No-wash, two-step workflow
Throughput	384-well plate format, quantify up to 10 unique analytes per well and 175 samples in duplicate per plate
Panel offering	Select from pre-designed or custom panels from over 800 antibody pairs in FirePlex portfolio
Sample input and compatibility	6.25 µl input of plasma, serum, or cell culture supernatant
Dynamic range	3-4 logs
Sensitivity	Average 1-100 pg/ml (*analyte-dependent)
Precision	<15% intra-plate CVs, 70-130% sample recovery
Readout and analysis	Scanned on high-content imagers (<20 min scan time/plate); data analysis using FirePlex Analysis Workbench

Assay workflow

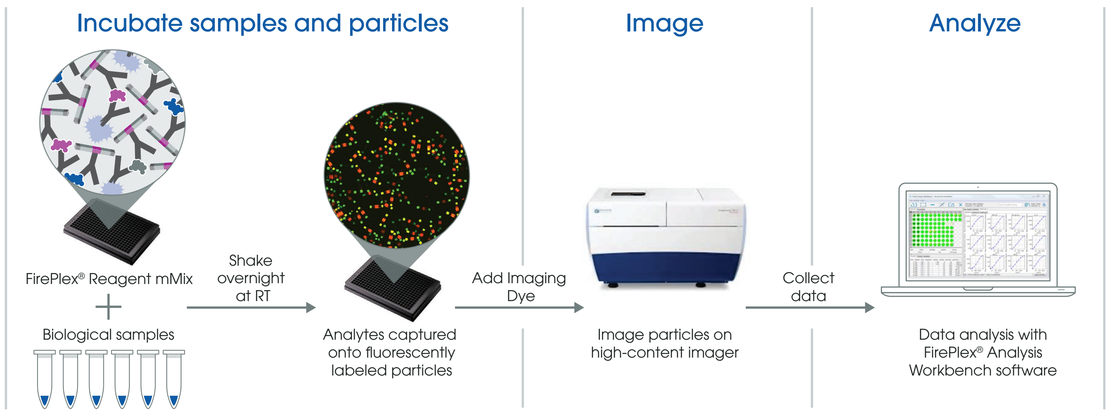
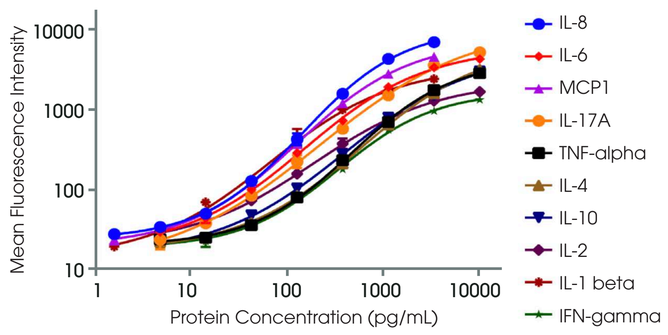


Figure 2. To capture analytes onto FirePlex particles, biological samples are added to a 384-well imaging plate and incubated with the FirePlex-HT Reagent Mix overnight. Subsequently, an Imaging Dye is added and plates are scanned on high-content imagers (refer Figure 6D for list of currently validated high-content imagers). The FirePlex Analysis Workbench software generates standard curves, and quantifies analytes of interest directly from image files.

Assay performance

A



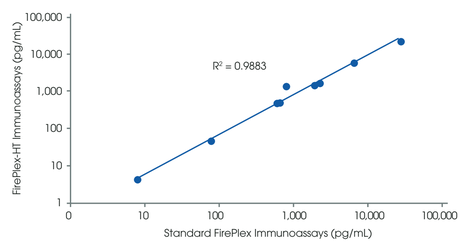
B

Human analytes	Sensitivity (pg/mL)	Dynamic range (pg/mL)	Inter-well CV (%)
IFN-gamma	72.48	123.4 - 10,000	11.50%
IL-1 beta	4.54	4.5 - 3,333	16.10%
IL-2	13.51	13.7 - 10,000	5.20%
IL-4	19.96	41.1 - 10,000	5.90%
IL-6	3.06	4.5 - 10,000	8.40%
IL-8	5.72	13.7 - 3,333	7.40%
IL-10	22.75	41.1-10,000	9.00%
IL-17A	7.04	13.7-10,000	13.40%
MCP1	2.43	4.5 - 3,333	13.30%
TNF-alpha	18.03	41.1 - 10,000	4.70%
IFN-gamma	72.48	123.4 - 10,000	11.50%

Figure 3. **A**. Standard curve analysis of a human 10-plex panel (ab234897) analyzed with the FirePlex-HT immunoassay platform. Analyses were performed using the TTP Labtech Mirrorball® high-content imager. **B**. Analyte performance of human cytokines evaluated with FirePlex-HT. For each analyte, the sensitivity and dynamic range are presented. Inter-well variation for each was also determined by calculating the CV between two independent wells of the standard curve.

FirePlex immunoassay platform comparisons

Figure 4. Correlation plot comparing the performance of the standard and high-throughput FirePlex immunoassays. Standard curve analyses were performed with a mouse 9-plex panel, using stimulated mouse splenocytes cell culture supernatantsamples. Samples were analyzed using either the standard-throughput FirePlex Immunoassays (Standard FirePlex), or the high-throughput FirePlex Immunoassays (FirePlex-HT).



Assay automation

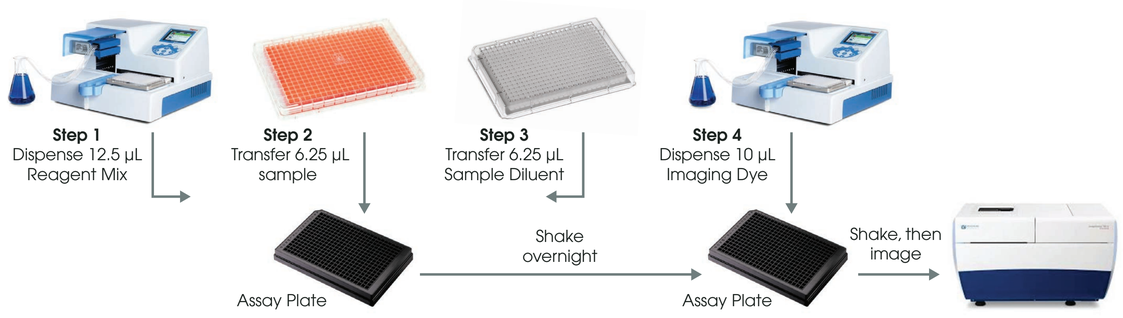
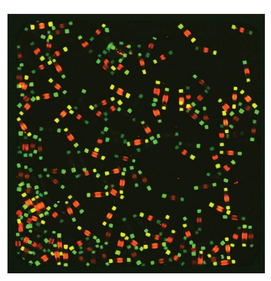


Figure 5. Example of an automated workflow for the FirePlex-HT Immunoassay. The Reagent Mix is dispensed into the Assay Plate using a ThermoFisher™ Multidrop™ Combi (Step 1), followed by transfer of biological samples (Step 2) and Assay Diluent (Step 3) into the Assay Plate using a liquid handler with a 384 channel pipette head. Samples are incubated overnight at room temperature, followed by addition of the Imaging Dye into the Assay Plate using a Multidrop™ Combi (Step 4) and image acquisition with a High-Content Imager (Step 5).

Data collection with high-content imagers

A



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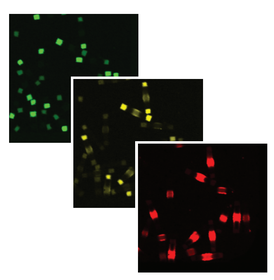

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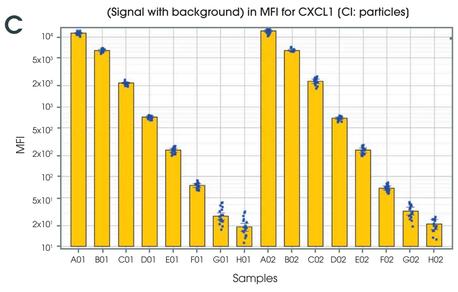
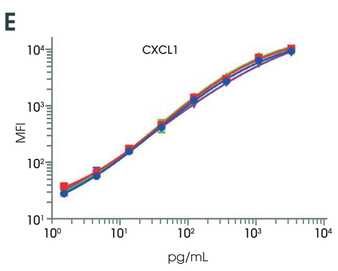


D

Field of view	Detection	Color channel	Excitation	Emission
Entire area of single well on a 384-plate with 4X objective	16-bits CMOS Camera(s)	Green	440-500 nm	500-535 nm
		Yellow	440-500 nm	570-630 nm
		Red	490-560 nm	650-760 nm

Imager Class	Make & Model
Microscope	Molecular Devices - ImageXpress Micro Confocal
	PerkinElmer - Opera Phenix
	GE - IN Cell Analyzer 6000
	GE - IN Cell Analyzer 2200
	Yokogawa - Cell Voyager 7000s
	Yokogawa - Cell Voyager 8000
Plate Scanning Cytometer	TTP Labtech - Mirrorball

E



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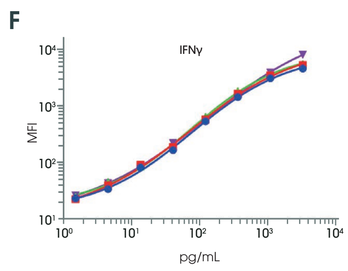

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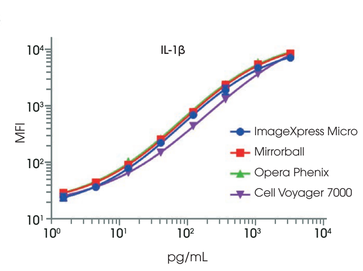

Figure 6. **A-B**. Varying levels of green and yellow fluorescent dyes are used for particle identity barcoding, and a red fluorescent dye is used for protein analyte quantification. Three images are captured per well, with approximately 20 particles analyzed per analyte using the FirePlex Analysis Workbench software. C. Representative graph demonstrating red fluorescent intensity levels automatically output from scanned wells. D. List of high-content imagers currently validated with FirePlex-HT immunoassays, and their required specifications. E-G. Representative plots of an eight-point standard curve analysis for CXCL1 (E), IFNγ (F), and IL-1β (G) scanned on the indicated high-content imagers and analyzed with the FirePlex Analysis Workbench software.

Data analysis using FirePlex Analysis Workbench

Figure 7. The FirePlex Analysis Workbench is an integrated and user-friendly data analysis tool that decodes image files collected from high-content imagers into analyte-specific data. Following the import of images into the software, users define wells containing the standard curve and the software performs curve fitting and interpolates analyte concentrations for each biological sample. Analyzed datasets can be exported in multiple formats that are compatible with other data analysis software packages, and data figures can be exported in high-quality format for use in publications. Analysis features include standard curve analyses, differential expression, box plots, and bar graphs.