Accelerating high-throughput screening using FirePlex®-384 multiplexing technology with high-content imaging and laboratory automation

D. Schwartz, B. Heinrich, W. Austin, C. Rafferty, L. To, and P. Wylie
Abcam Inc. 152 Grover Street, Suite 100, Waltham, MA 02453

Introduction

FirePlex-384 is a new multiplex assay platform integrating the rapid data harvesting power of multiplexing with standard High-Content Imaging instrumentation in an easy to use automatable high-throughput platform.

High-throughput screening (HTS) technologies play an essential role in drug discovery by rapidly querying large numbers of biological samples for biomarkers or compound effects to guide development decisions. Multiplexing is a highly effective tool for significantly increasing screening throughput and efficiency, but technically challenging and expensive to implement into HTS workflows. Closed multiplexing platforms with expensive dedicated instrumentation and complicated workflows also present obstacles for adoption.

FirePlex-384 multiplex platform addresses these obstacles using patented FirePlex hydrogel particle technology. The three-region encoded particle design provides sensitive and reproducible 10 analyte in-well multiplexing for a range of assay applications. Assay data acquisition is conducted on standard High-Content Imagers, and data analysis is achieved with the FirePlex Analysis Workbench software, eliminating the need for purchase of an expensive dedicated instrument and software licenses. Easy to use optional two-step workflow or no-wash assay workflows limit hands-on time and are compatible with high-throughput liquid handling robotic laboratory automation. FirePlex-384 catalogue immunoassays offer up to 10 multiplexed protein analytes on pre-designed content panels or flexible Mix & Match pools for custom configurations.

FirePlex-384 can be deployed existing High-Content Imaging and laboratory robotic instrumentation to provide true multiplexing in a sensitive, reliable, easy to use and economic platform.

Contact us to learn more about FirePlex-384

FirePlex particle overview

At the heart of the FirePlex-384 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.

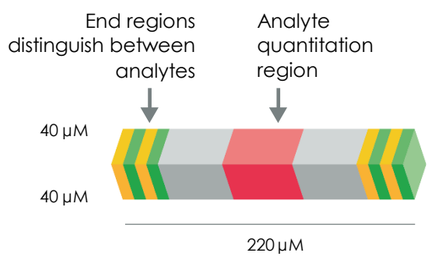


Figure 1. FirePlex particles provide optimal thermodynamics, detection directly from biofluids and is decoded by our integrated analysis software.

Assay specifications

Workflow format	No-wash or two-step workflow
Throughput	384-well plate format, quantify up to 10 unique analytes per well and 175 samples in duplicate per plate, including controls and standards.
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

FirePlex-384 simplified no-wash assay workflow

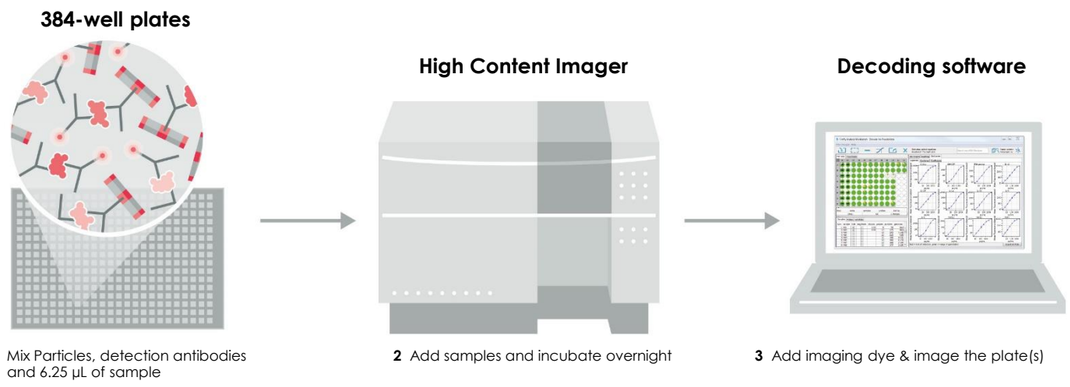
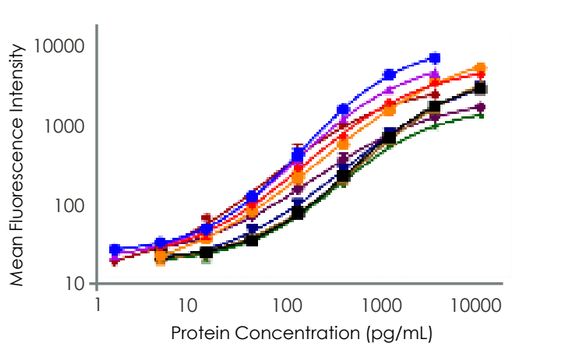


Figure 2. To capture analytes onto FirePlex particles, biological samples are added to a 384-well imaging plate and incubated with the FirePlex-384 Reagent Mix overnight. Subsequently, an Imaging Dye is added and plates are scanned on high-content imagers (refer to table on right for list of currently 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%

Figure 3. A. Standard curve analysis of a human 10-plex panel (ab234897) analyzed with the FirePlex-384 immunoassay platform. Analyses were performed using the TTP Labtech Mirrorball® high-content imager. B. Analyte performance of human cytokines evaluated with FirePlex-384. 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.

Assay automation

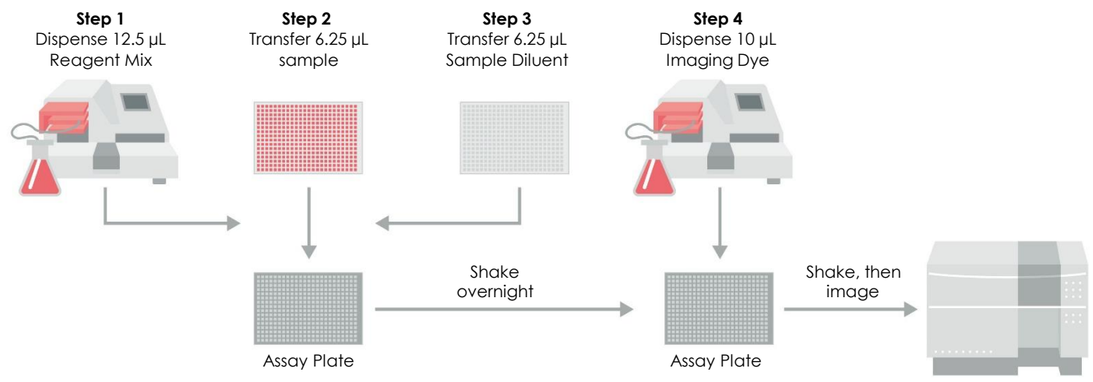
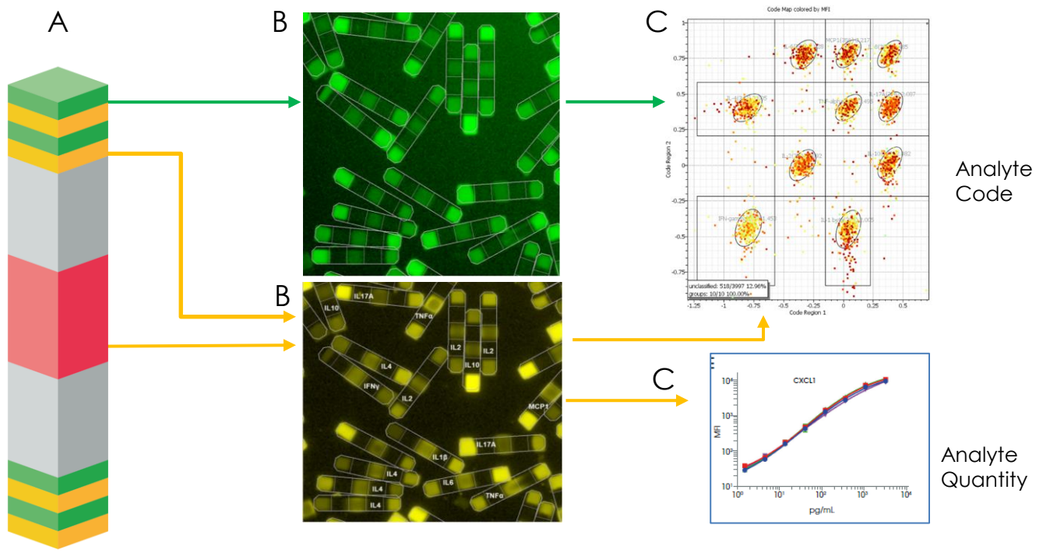


Figure 4. Example of an automated workflow for the FirePlex-384 Immunoassay. The Reagent Mix is dispensed into the Assay Plate using a ThermoFisher™ Multidrop™ Combi (Step 1), followed by transfer of biological samples (Step 2) and Assay Diluent (Step 3) into the Assay Plate using a liquid handler with 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



D

Field of View	Detection	Color Channel	Excitation	Emission
Entire single well area of 384-well plate at 4X objective	16-Bit CMOS Camera	Green	440-500 nm	500-535 nm
		Yellow	440-500 nm	570-630 nm

Imager Class	Make	Model
Microscope	Molecular Devices	Image Express Pico, Micro Confocal, Micro 4
	Perkin Elmer	Opera Phenix Operetta CLS
	GE	In Cell Analyzer 6000 In Cell Analyzer 2200
	Yokagawa	Cell Voyager 7000s Cell Voyager 8000
	Nexcelom	Celigo
	BioTek	Cytation 5
	Thermo	Cytation CX7 (or other 16-bit/CMOS)
	Tecan	Spark
Plate Scanning Cytometer	TTP Labtech	Mirrorball

Figure 5. A. Varying intensities of green and yellow fluorescent dyes are used for particle identity barcoding, yellow dye co-locally separate on the particle from the barcoding region is used for analyte quantitation. B. Two images are captured per well, with approximately 20 particles analyzed per analyte using the FirePlex Analysis Workbench software. C. Representative graph demonstrating analyte fluorescent intensity levels automatically output from scanned wells along with analyte standard curves. D. List of high-content imagers currently validated with FirePlex-384 immunoassays, and their required specifications.

Data analysis using FirePlex Analysis Workbench

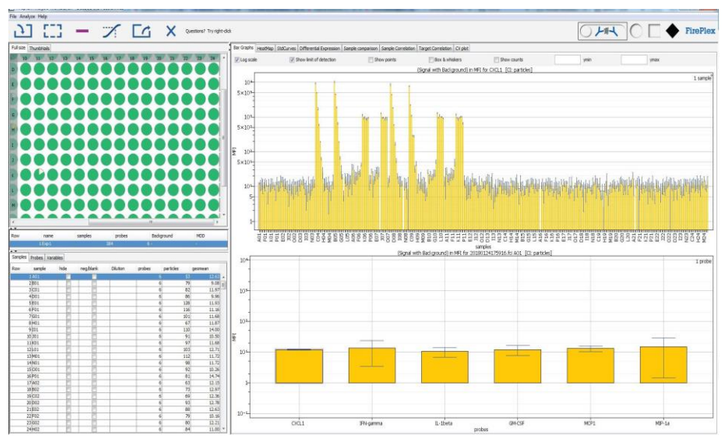


Figure 6. The FirePlex Analysis Workbench is an integrated and user-friendly data analysis tool that decodes image files collected from high-content imagers into analyte-specific data. Following the import of images into the software, users define wells containing the standard curve, and the software 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.

Conclusion

The merging of high-throughput systems with multiplexing assay technologies provides a method for biomarker efficiently screening very large numbers of biological samples. Combining of these two technologies is complicated and expensive. FirePlex-384 addresses the need for a sensitive and reliable multiplex assay platform that is compatible with high throughput workflows, accelerating biomarker or compound screening for drug discovery easily and inexpensively.