

Introducing FirePlex®-HT: High-throughput, multiplex immunoassays using FirePlex® Particle Technology

A. Perea, B. Heinrich, W. Austin, C. Rafferty, M. Camilleri, L. To, E. Atabakhsh, and D. Pregibon
Abcam Inc. One Kendall Square, Building 200, Cambridge, MA 02139. FirePlex@abcam.com

To address the need for rapid and sensitive quantitation of protein 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 sample 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.

Assay Specifications

- **Workflow format:** No-wash assay, 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 250 antibody pairs in FirePlex portfolio
- **Sample input and compatibility:** 6.25 µl input of plasma, serum, or cell culture supernatant
- **Dynamic range and sensitivity:** 3-4 logs dynamic range, and average 1-100 pg/ml sensitivity* (*analyte-dependent)
- **Precision:** <15% intra-plate CVs, 70-130% sample recovery
- **Readout and analysis:** Scanned on high-content imagers (<20min scan time/plate); data analysis using FirePlex Analysis Workbench

Assay Workflow

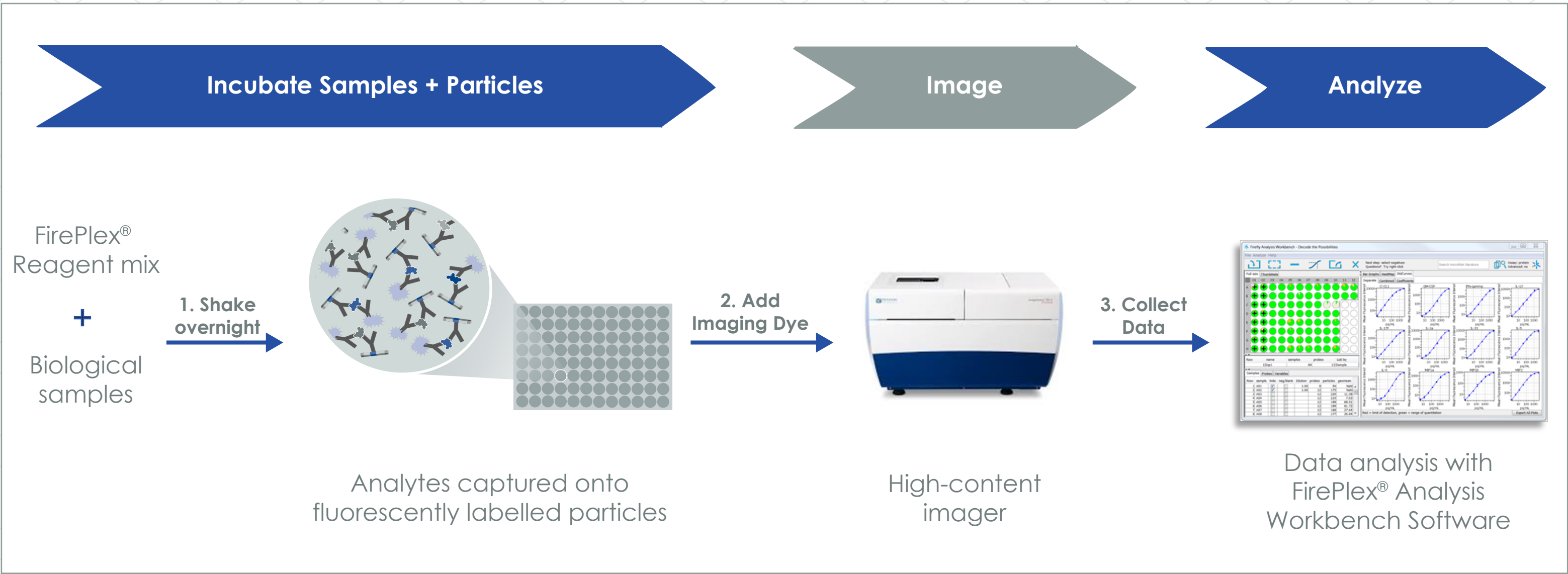


Figure 1. To capture analytes onto FirePlex particles, biological samples are added to a 384-well imaging plate and incubated with the FirePlex-HT Reagent Mix overnight. Subsequently, an Imaging Dye is added and plates are scanned on high-content imagers (refer Figure 6D for list of currently validated high-content imagers). The FirePlex Analysis Workbench software generates standard curves, and quantifies analytes of interest directly from image files.

Data Collection with High-Content Imagers

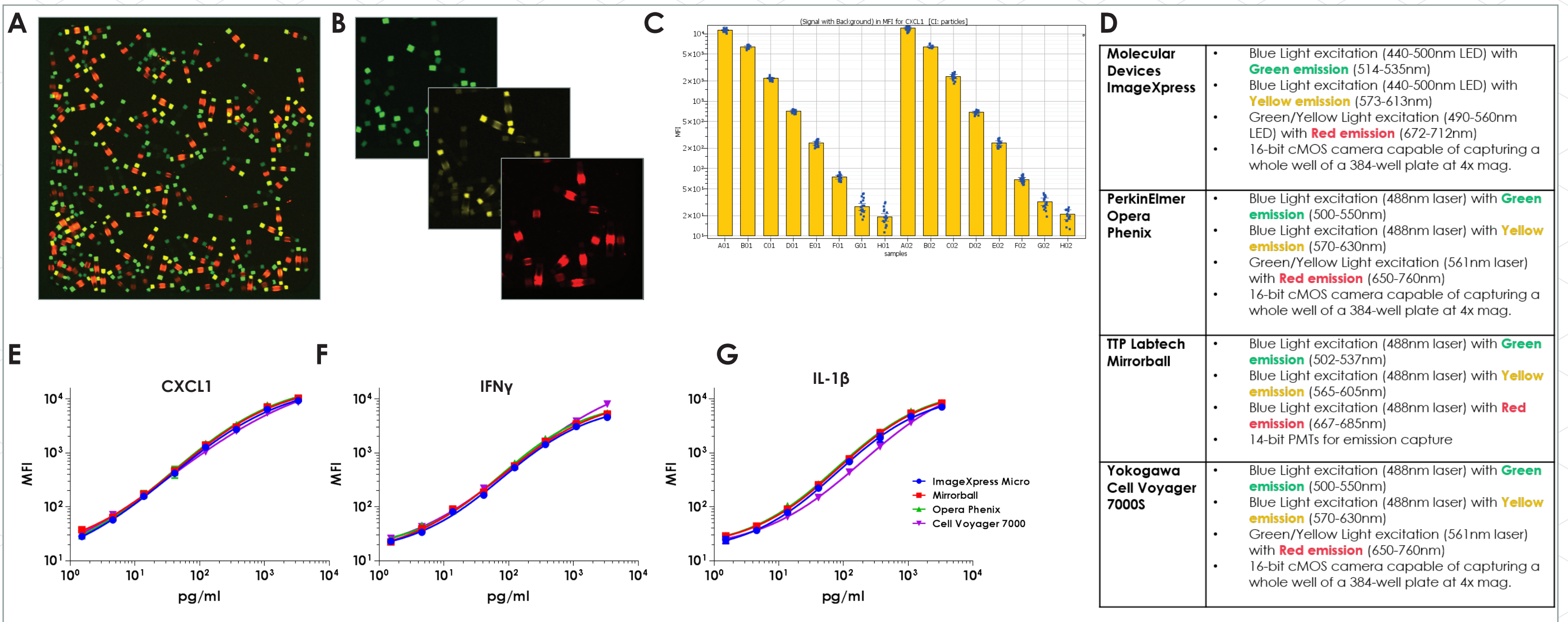


Figure 6. A-B. Varying levels of green and yellow fluorescent dyes are used for particle identity barcoding, and a red fluorescent dye is used for protein analyte quantification. Three images are captured per well, with approximately 20 particles analyzed per analyte using the FirePlex Analysis Workbench 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 Molecular Devices ImageXpress Micro, PerkinElmer Opera Phenix, TTP Labtech mirrorball, and Yokogawa Cell Voyager 7000 high-content imagers and analyzed with the FirePlex Analysis Workbench software.

Assay Performance

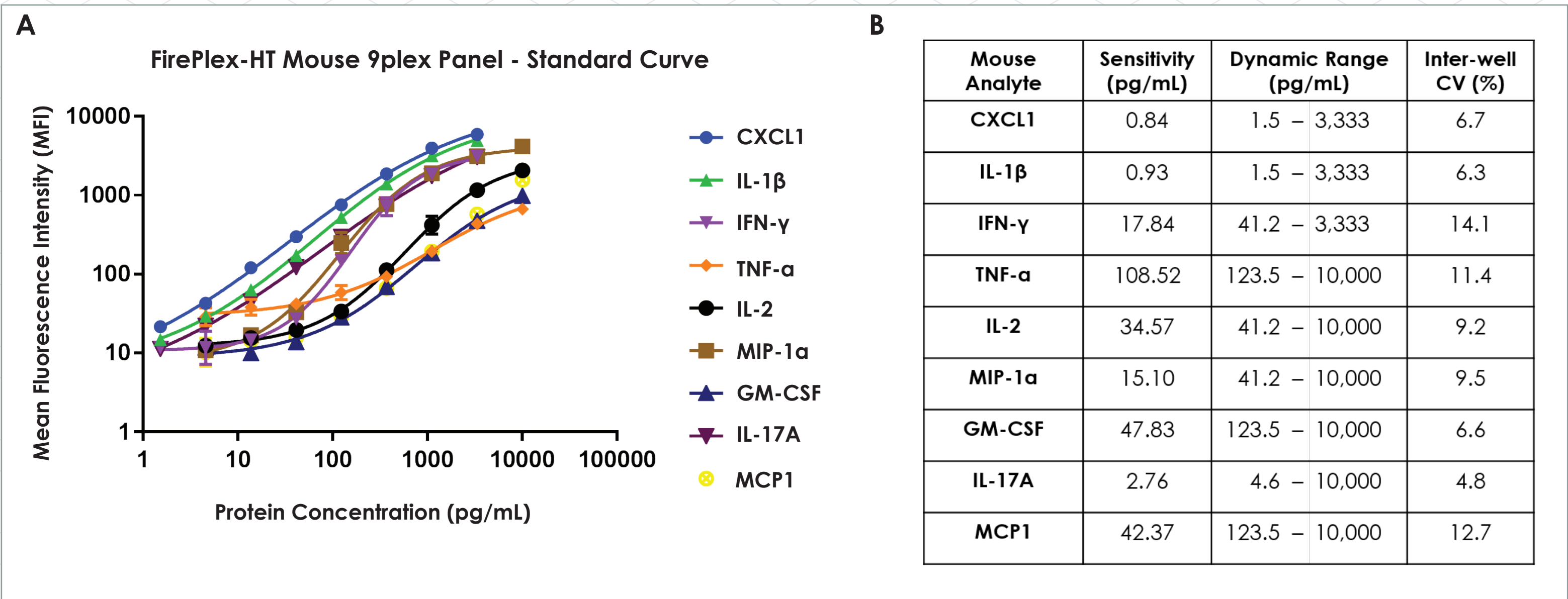


Figure 2. A. Standard curve analysis of a mouse 9plex panel analyzed with the FirePlex-HT immunoassay platform. Data are plotted as background-subtracted mean fluorescence intensities (MFI) for each analyte, +/- the standard deviation of the mean. Analyses were performed using the TTP Labtech Mirrorball high-content imager. B. Analyte performance of mouse 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.

Assay Automation



Figure 5. A. Example of an automated workflow for the FirePlex-HT Immunoassay. The Reaction 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). B. Representative dataset from a FirePlex-HT immunoassay experiment run with an automated workflow. Data are interpolated concentrations of CXCL1 in untreated and treated cell culture supernatants.