abcam

Technical note

FirePlex®-96 Immunoassays

Multiplex protein quantitation using FirePlex® particle technology

www.abcam.com/FirePlex

Multiplex biomarker profiling

FirePlex®-96 immunoassays offer comprehensive biomarker profiling using innovative FirePlex particle technology, enabling high-performance multiplexing capabilities. This technology allows you to identify and quantitate up to 70 analytes within a single 12.5 µL sample, directly from biological fluids, tissues, or cell suspensions.

A 96-well plate immunoassay format enables high-throughput screening on a standard flow cytometer for fast and easy readout. Our recombinant monoclonal antibody pairs offer superior sensitivity and specificity, detecting analytes as low as 0.5 pg/mL over a 5-log dynamic range.

Choose from our broad range of pre-designed discovery panels, cytokine focus panels, toxicity-screening panels or customize your own personalized panel to target your specific analytes of interest. Complex data analysis is made simple with our free-of-charge integrated FirePlex Analysis Workbench software, which automatically calculates results and generates publication-ready figures. Alternatively, you can leave the work to us and let our full-service team of experts analyze your samples from start to finish.

FirePlex particle technology

The unique architecture of FirePlex particles offers several advantages over standard bead-based assays. The porous hydrogel composition enables analyte capture throughout the particle, providing a broader dynamic range than the limited surface capture of rigid microspheres.

In addition, the innovative rod-shaped design features three distinct functional regions that allow FirePlex particles to distinguish and quantify up to 70 different analytes simultaneously in a single well (Figure 1). Functional regions are separated by inert regions to minimize code-bleed, providing superior sensitivity (Figure 1).

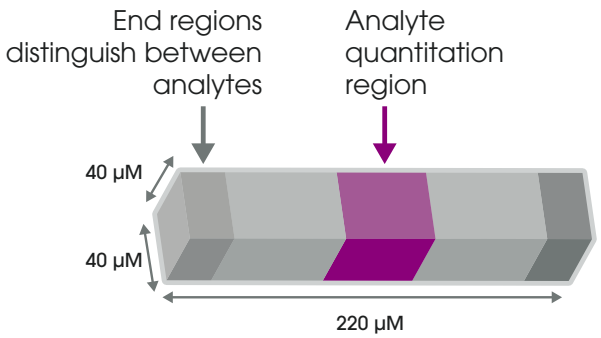


Figure 1. FirePlex particle design. FirePlex particles contain three functional regions separated by two inert spacer regions. The two terminal regions serve for particle encoding, which is achieved using varying degrees of green and yellow fluorescent dyes. The center region of the particles contains the analyte quantitation region, where analyte-specific capture antibodies are conjugated to the particles, and a red fluorescent dye is used for analyte quantitation. The two spacer regions serve to reduce code interference while particles are being analyzed on a flow cytometer.

Immunoassay workflow

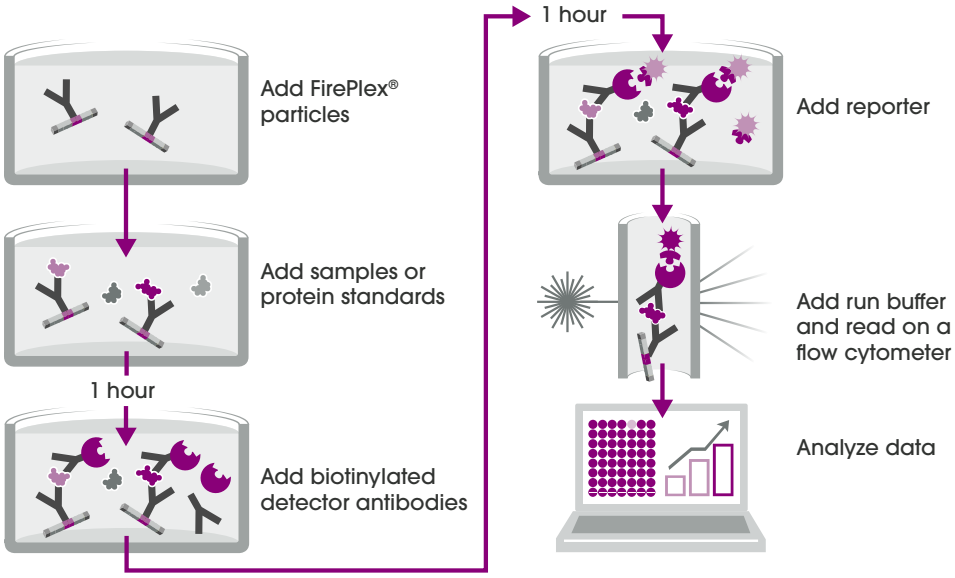


Figure 2. FirePlex-96 immunoassay workflow. Assay run-time is 2.5 hours -- wash steps streamlined with vacuum manifold and microplate shaker. Particles are then scanned in 96-well filter plate format on a compatible flow cytometer, yielding over 7,000 data points per plate when analyzing a 70-plex particle panel. The integrated FirePlex Analysis Workbench software easily decodes the data for you, plots standard curves, and delivers results.

High-performance multiplexing

FirePlex immunoassays use Abcam's high-performance recombinant rabbit monoclonal antibodies, which are paired to reduce cross-reactivity and improve performance capabilities:

- only 12.5 µL of sample input required
- <1 pg/mL sensitivity (analyte dependent)
- reliable detection from 0.5 pg/mL to 30,000 pg/mL
- up to 5-logs dynamic range (Figure 3)
- intra-assay CV <10%
- inter-assay CV <15%

Compared with competitors, FirePlex's superior sensitivity allows for detection and quantitation of analytes at very low concentrations, with a median assay sensitivity of 0.5 pg/mL. Meanwhile, the broad dynamic range of FirePlex immunoassays yields better accuracy and precision for measuring analytes at the lower and upper ends of concentration ranges (Table 1).

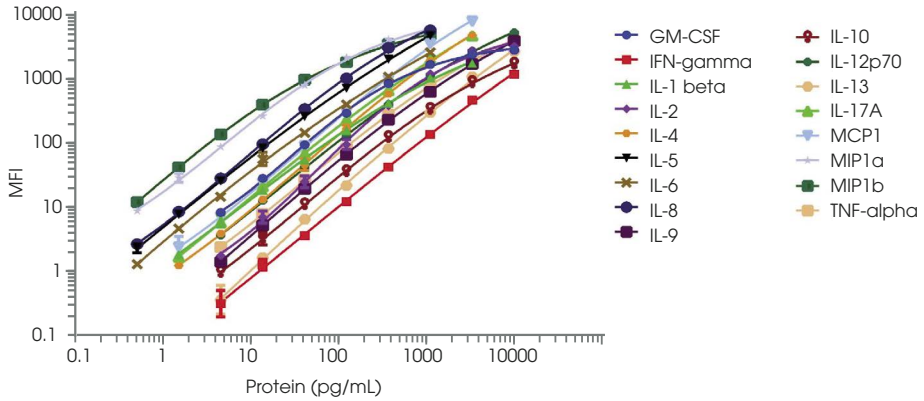


Figure 3. Broad dynamic range. Eight-point standard curve analysis for the FirePlex human 17-plex key cytokine panel (ab243549).

Analyte	FirePlex Immunoassay		Leading competitor
	Dynamic Range (pg/mL)	Sensitivity (pg/mL)	Sensitivity (pg/mL)
GM-CSF	4.57-10,000	0.61	0.2-4.1
IFN-γ	4.57-10,000	1.59	0.4-6.4
IL-1β	1.52-3,333	0.31	0.6-3.6
IL-2	4.57-10,000	1.16	0.3-1.6
IL-4	1.52-3,333	0.42	2.2-20.2
IL-5	0.51-1,111	0.1	0.7-32.4
IL-6	0.51-1,111	0.16	1.8-3.3
IL-8	0.51-1,111	0.17	1.6-9
IL-9	4.57-10,000	0.71	0.7-14
Il-10	4.57-10,000	1.24	0.5-1.5
IL-12p70	4.57-10,000	0.79	1.7-2.9
IL-13	4.57-10,000	3.18	0.7-1.8
IL-17A	1.52-2,333	0.36	2.5-8.7
MCP1	1.52-3,333	0.26	1.1-9.9
MIP1a	0.51-1,111	0.08	1.6-16.2
MIP1b	0.51-1,111	0.17	2.4-16.2
TNF-α	4.57-10,000	1.78	1.2-6

Table 1. Superior sensitivity. Sensitivity and dynamic range of FirePlex human 17-plex key cytokine panel (ab229791) compared to the competitor.
Highlighted cells indicate where FirePlex antibody pairs demonstrate higher sensitivities to the published sensitivities for other bead-based multiplex immunoassay platforms.

Analyze diverse biofluids from raw samples

FirePlex immunoassay antibody pairs are validated on a broad range of biofluids. Human, mouse, and rat panels are validated in plasma (EDTA, heparin, citrate), serum, urine, and cell culture supernatant. Human analytes are additionally validated in saliva, milk (defatted), synovial fluid, bronchioalveolar lavage (BAL), and cerebrospinal fluid (CSF) (Figure 4).

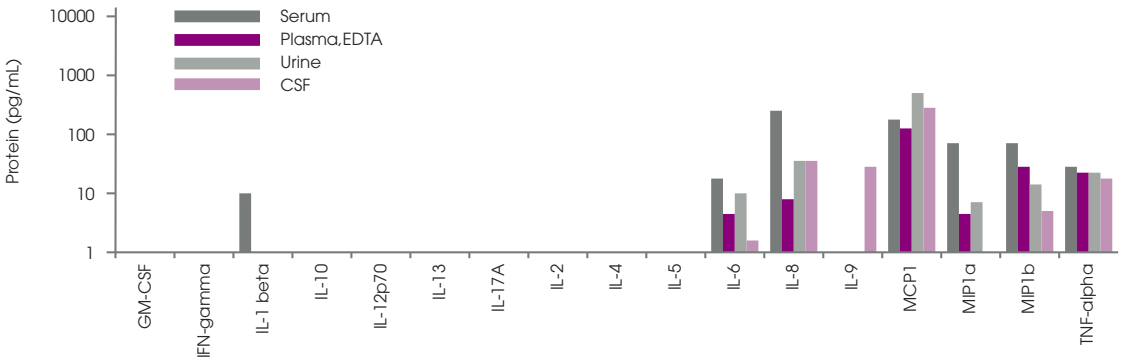


Figure 4. Sample type validation. Validation of antibody pairs in the Human 17-plex Key Cytokine panel in pooled human serum, plasma, urine, and CSF.

Throughout pair and assay development, FirePlex panels are tested in clinically relevant biological samples to confirm that results are consistent with the literature. For example, pro-inflammatory cytokines are undetected in normal healthy human serum or plasma, whereas they are elevated in PHA stimulated PBMCs (Figure 5).

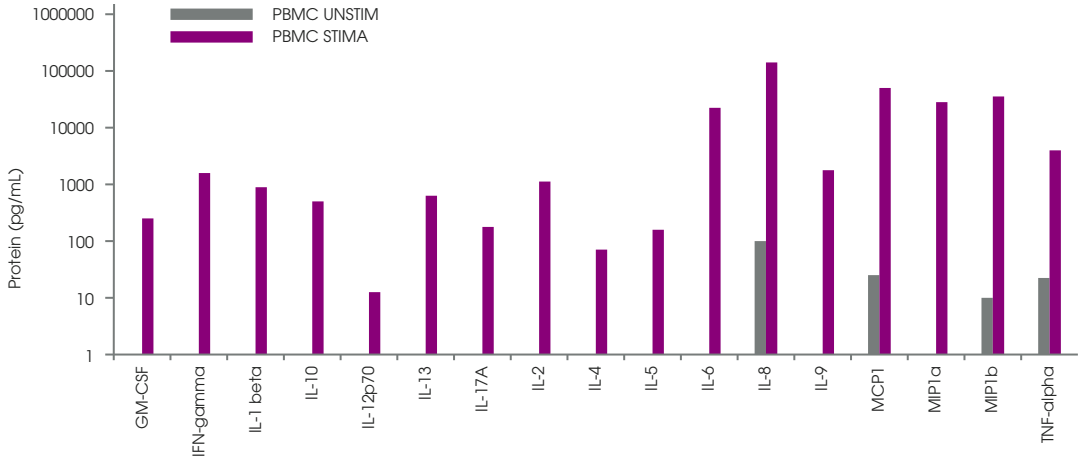
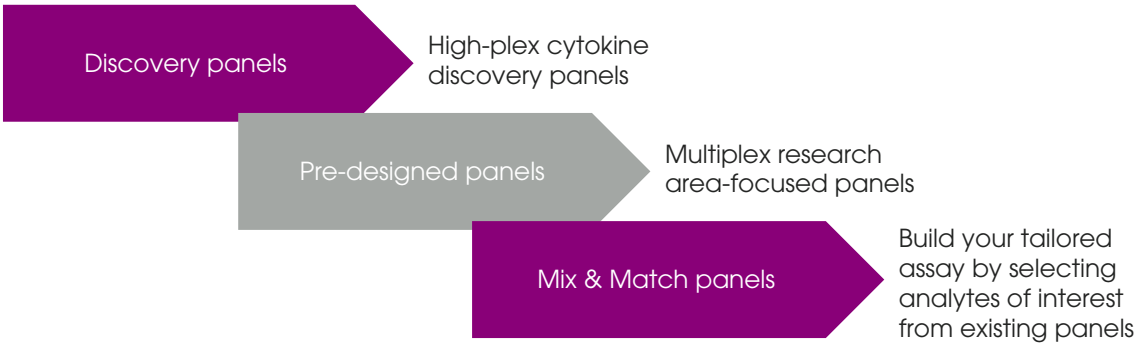


Figure 5. Pro-Inflammatory cytokine validation. Validation of antibody pairs in the Human 17-plex Key Cytokine panel with unstimulated and stimulated human PBMCs.

Flexible panel and assay choices

Choose from our multiple panel and assay offerings to fit your research needs.



Discovery panels

For discovery projects and in-depth studies, mid- and large- plex panels offer 36 and 70 analyte compilations for extensive biomarker analysis in human samples.

* 70-plex discovery panels are available as projects through the FirePlex Service Lab. Contact us at go.myabcam.com/fireplex-online-enquiry for more information.

Pre-designed panels

Pre-designed panels offer a plug-and-play option for fast and easy quantitation of key cytokines and inflammatory markers in human, mouse, and rat. The new mouse kidney toxicity panels provide high-throughput screening tools for monitoring organ toxicity in preclinical animal models during the drug development pipeline.

Human panels

Discovery	Please see the flyer for the full list of 70 analytes	ab243551
Inflammation	IL-1β, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, MCP1, TNF-α	ab243550
Key cytokine	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-17A, GM-CSF, IFN-γ, MCP1, MIP1-α, MIP1-β, TNF-α	ab243549

Mouse panels

Discovery	Please see the flyer for the full list of analytes	ab243554
Key cytokine	CXCL1, GM-CSF, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17A, MCP1, MIP1α, MIP1β, TNFα	ab235656
Inflammation	CXCL1, IFN Gamma, IL-1 beta, IL-6, IL-10, IL-12p70, MCP1, TNF alpha	ab235659
Kidney toxicity	Clusterin, Cystatin C, KIM-1, Lipocalin-2, Osteopontin	ab235661

Rat panels

Key cytokine	CXCL1, GM-CSF, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17A, MCP1, MIP1α, MIP1β, TNFα	ab235662

Non-Human Primate (NHP) panels

Key cytokine	GM-CSF, IFNγ, IL-1β, IL-10, IL-12p70, IL-13, IL-17A, IL-2, IL-4, IL-5, IL-6, IL-8, MCP-1, MIP1α, MIP1β, TNFα	ab239455

For tissue lysate preparation, we have a FirePlex intracellular immunoassay core kit (ab239455).

Mix & Match panels

Design your own ideal multiplex panel from pre-developed optimized analyte pools to fit your specific research goals. Choose from over 150 analytes with full flexibility to mix and match up to 35 different analytes in one panel. The FirePlex Assay Builder makes assembling your custom panel easy. Personalizing your own panel is an accessible and affordable option, with short lead times and small order minimums.
Contact us at go.myabcam.com/fireplex-mix-and-match

Free integrated bioinformatics tools for panel design and data analysis

FirePlex Mix and Match Assay Designer

This assay designer tool (Figure 6) allows you to select analytes of interest from existing panels to build the assay that best meets your needs:

– Pick a perfect panel from a list of ever-expanding antibody pairs
– Select up to 35 analytes for FirePlex-96
– Filter by species: Human, Mouse

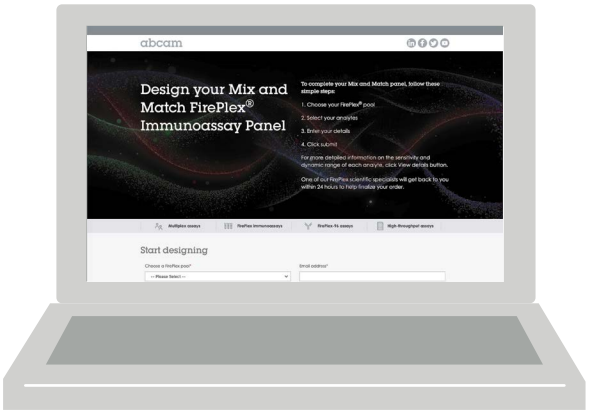


Figure 6. FirePlex Assay Designer.

FirePlex Analysis Workbench

The FirePlex Analysis Workbench software provides fast and easy-to-use data analysis, free of charge. The sophisticated software decodes flow cytometer .fcs files, performs standard curve analysis, quantitates analytes, and generates publication-quality plots and graphs, which can be easily exported for manuscript preparation (Figure 7).

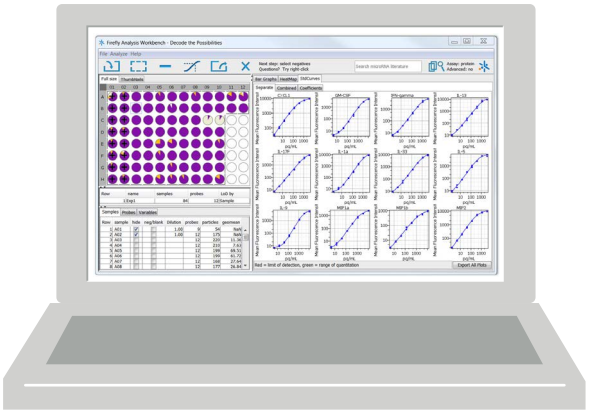


Figure 7. FirePlex Analysis Workbench software.

Compatible flow cytometers

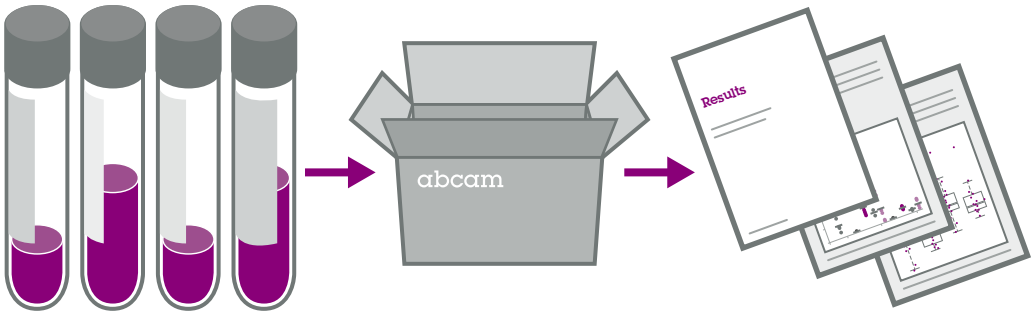
FirePlex immunoassays can be conveniently run on your own existing flow cytometer, eliminating the need to purchase dedicated equipment. FirePlex particles are validated for use with the flow cytometers fitted with green, yellow and red detectors (See Appendix).

Our Cytometer Setup kit (ab211043) can be used to ensure cytometer is compatible and has appropriate settings.

Sample profiling services

Send us your samples for profiling and let us do the work. Our team of FirePlex experts* offers full support, from generating hypotheses to delivering results. You can confidently entrust your research to our skilled scientists with a wealth of experience accumulated over testing 10,000 customer samples. Results, along with publication-ready charts, tables, and raw data files, are delivered within approximately 15 business days.

*Contact our experts at go.myabcam.com/fireplex-online-enquiry



Performance guarantee

Our team works hard to develop, deliver and guarantee the highest quality products, with rigorous validation, performance and benchmark testing of immunoassay workflows and panel designs.

All antibody pairs are extensively tested for cross-reactivity of capture and detector antibodies, as well as for protein interference. Combinatorial tests confirm that individual FirePlex immunoassays retain specificity in multiplex with other assays and confirm the specificity of antibody pairs against the analyte of interest.

Stringent quality control testing ensures the highest performance standards for assays and reagents. Abcam's human and mouse recombinant monoclonal antibodies are paired for excellent lot-to-lot reproducibility and sub-picogram/mL detection sensitivity. Accelerated and real-time course stability studies confirm reagent stability. Original and subsequent lots are retained as references for subsequent assay production.

When choosing FirePlex immunoassays for your research, you can be confident in their performance ensured by comprehensive validation.

References

1. Lee, J. W. *et al.* Fit-for-purpose method development and validation for successful biomarker measurement. in *Pharmaceutical Research* **23**, 312–328 (2006).
2. Jani, D. *et al.* Recommendations for Use and Fit-for-Purpose Validation of Biomarker Multiplex Ligand Binding Assays in Drug Development. *AAPS* J. **18**, 1–14 (2016).
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4. Ohl, K. & Tenbrock, K. Inflammatory cytokines in systemic lupus erythematosus. *J. Biomed. Biotechnol.* **2011**, 432595 (2011).
5. McInnes, I. B. & Schett, G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat. Rev. Immunol.* **7**, 429–442 (2007).

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Appendix

FirePlex particles are validated for use with flow cytometers fitted with green, yellow, and red detectors. The following excitation and emission settings are required:

Fluorochrome	PMT Channel	Excitation	Emission
FITC - Green	FL1	488 nm	530/30 nm
PE - Yellow	FL2	488 nm	575/25 nm
PerCP - Cy5.5	FL3	488 nm	695/40 nm