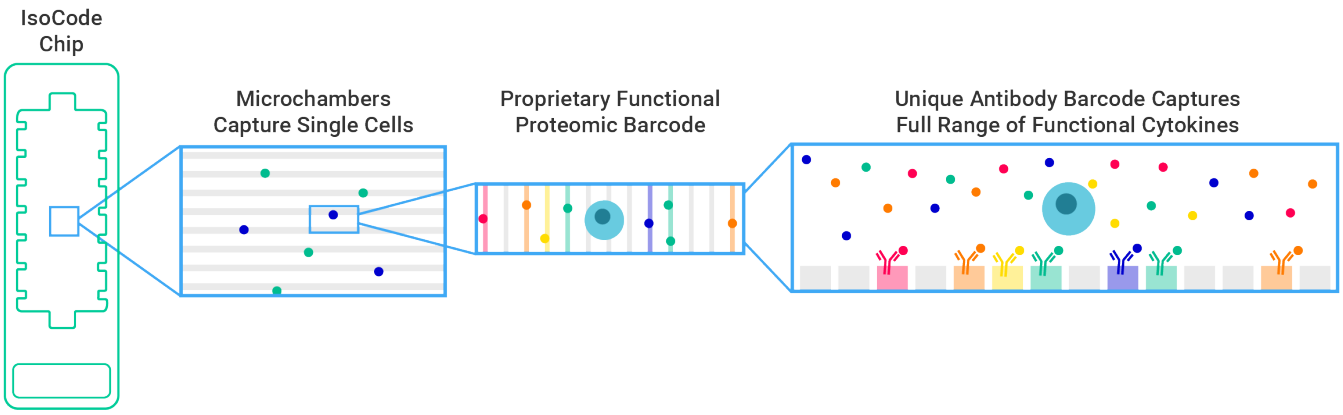
Bruker Grant Support Package

Bruker's Platforms

- *Are the only systems that can perform automated highly multiplexed live single cell secretome analysis, single cell highly multiplexed phosphoproteomic analysis, as well as bulk/population protein analysis all on one simple platform.*
- *Our IsoCode Single-Cell Secretome technology is the only system that, by utilizing an on-board CO_2 incubator, can provide highly multiplexed secreted protein profiles of up to 30+ different cytokines from 500-1500 live single cells.*
- *Our Single-Cell Signaling technology is the only system that can provide highly multiplexed intracellular phosphoprotein analysis of up to 15+ different phosphoproteins from single cell.*
- *Our CodePlex Secretome technology is the only system that can provide highly multiplexed bulk secreted protein analysis of up to 30+ different cytokines from very low sample volumes of 11 µl total (5.5 µL per replicate) in a completely automated manner.*
- *IsoSpeak is the only proteomics analysis software on the market that integrates with the instrument to provide automated data analysis and generate advanced 3D visualizations such as UMAP and t-SNE, with a guided user interface.*

Single-Cell Secretome



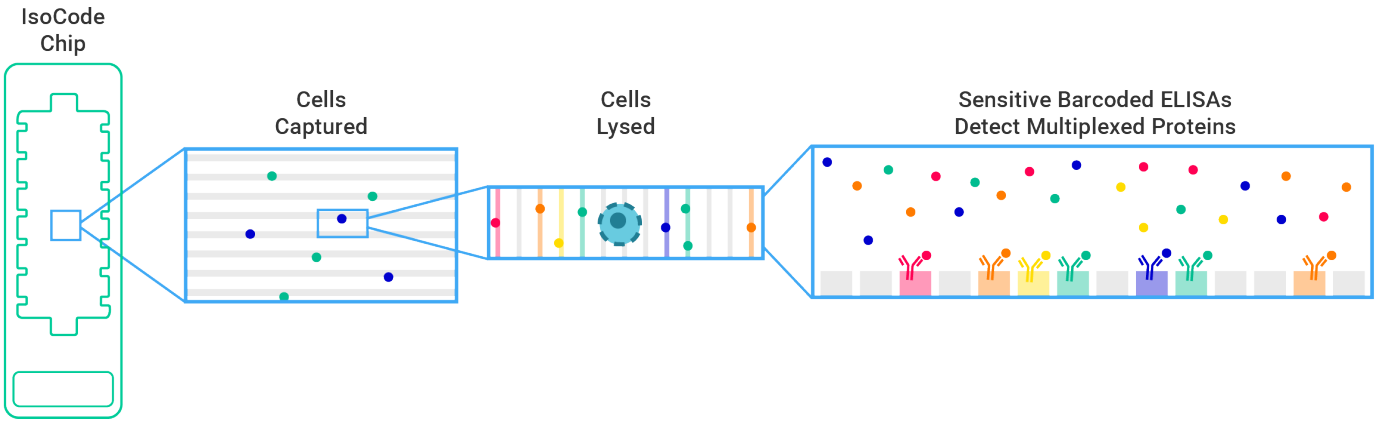
Secreted proteins, and in particular cytokines, have been shown to be key mediators of intercellular communication within the immune system. Due to the high degree of cellular heterogeneity, there is a need to measure an array of proteins from single cells in parallel to detect highly impactful polyfunctional cells (cells secreting two or more cytokines simultaneously). Many proteomic technologies have been developed to assess the immune response but are limited in their ability to measure true cell function. Bulk techniques average cytokine secretions across a population and are unable to specify what cytokines are produced by each heterogenous immune cell. Analysis of cellular RNA or surface phenotypes alone lack the ability to peer into the functional proteome. Other techniques are limited in the number of effector proteins able to be measured per cell, decreasing the ability to evaluate the full spectrum of T cell cytokine profile.

Analysis of the functional proteome through single-cell, highly multiplexed cytokine analysis is critical for identifying cells orchestrating the immune response. To address this gap, Bruker has developed the Single-Cell

Secretome platform, the only technology capable of detecting functional phenotypes for each immune cell by measuring secreted cytokines to reveal subsets of highly polyfunctional cells[1] that correlate to persistence, potency, durability, and other predictive biomarkers. Determining the quality of single immune cells by tracking polyfunctional response is necessary to understand positive patient outcomes to immune mediated therapies[2-7].

The Single-Cell Secretome workflow involves sample preparation and includes stimulation and/or enrichment of cells (when applicable, using validated protocols) to optimize analysis. After loading the sample, chips are placed into the instrument and the remainder of the workflow is fully automated. Microchambers on the chip capture individual cells for analysis. The cells are held at physiological conditions (37°C, 5% CO_2) throughout the assay to facilitate secretion of functional proteins. Proteomic analysis occurs using a sandwich ELISA method, where capture antibodies are bound to a glass slide in a specific spatial arrangement covering the microchambers. This antibody "barcode" allows for the highly multiplexed identification of secreted functional proteins within each microchamber, resulting in single-cell characterization across all microchambers in a parallelized manner. Imaging occurs after the automated ELISA protocol is complete and data related to intensity and spatial location of fluorophores are exported to the IsoSpeak software for analysis.

Single-Cell Signaling



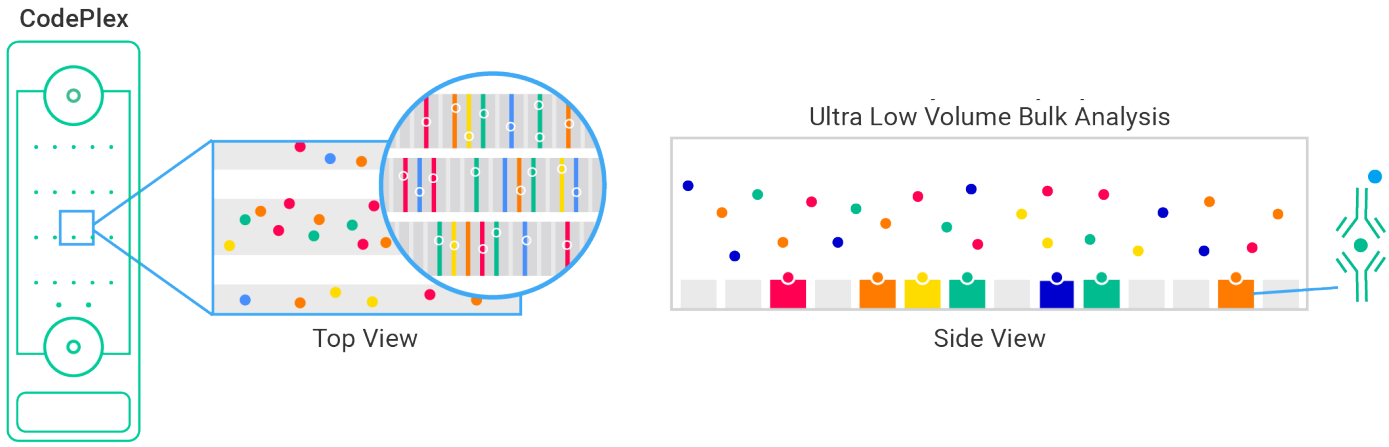
A major challenge in the field of cancer immunology is adaptive resistance which occurs when tumor cells rapidly develop resistance to targeted therapies by altering coordinated proteomic signals. To counteract this, it is necessary to identify the pathways involved in cell state transition toward drug resistant phenotypes. Measuring and identifying early activation pathways is critically important when tackling drug resistance. Detecting these key coordinated signals requires measuring simultaneous phosphoproteomic reactions from single cells in a highly multiplexed manner. Western blot misses these rare, coordinated signals as cells are averaged together, and single cell technologies are limited in their multiplexing capabilities (3-4 analytes per cell).

To address this gap, Bruker developed the Single-Cell Signaling platform which uniquely measures 15+ phosphoproteins from single cells simultaneously to reveal coordinated signals in rare subsets of tumor cells driving tumor resistance. Assessing the phosphoproteome at the single-cell level differentiates subpopulations and helps interrogate heterogeneity based on signaling networks. Furthermore, the highly multiplexed nature of

the assay provides a more complete understanding of intracellular signaling at the single-cell level than traditional methods. Revealing these coordinated signals can elucidate the impacts of therapies and mechanisms driving tumor resistance[8-10].

With the Single-Cell Signaling solution, cell samples that have been prepared (including target cell enrichment and/or stimulation, when applicable, using validated protocols) to optimize analysis are loaded onto the Single-Cell Signaling chip. After sample loading, chips are loaded into the instrument and the remainder of the workflow is fully automated. Microchambers on the chip capture individual cells for analysis. The cells are held at physiological conditions (37°C, 5% CO_2) throughout the assay and lysis occurs quickly to maintain signaling fidelity and capture of transient phosphoproteomes. Once sorted into microchambers, cells are lysed to release intracellular proteins for analysis. Phosphoproteomic analysis occurs using a sandwich ELISA method, where capture antibodies are bound to a glass slide in a specific spatial arrangement covering the microchambers. This antibody "barcode" allows for the highly multiplexed identification of intracellular phosphoproteins within each microchamber, resulting in single-cell characterization across all microchambers in a parallelized manner. Imaging occurs after the automated ELISA protocol is complete and data related to intensity and spatial location of fluorophores are exported to the IsoSpeak software for analysis.

CodePlex Secretome



Secreted proteins, and in particular cytokines, have been shown to be key mediators of intercellular communication within the immune system. Many analytical technologies have been developed to analyze the immune response but many measure limited numbers of cytokines and lack automation capabilities. Status quo highly multiplexed proteomic technologies require mundane and tedious workflow steps that require hours of hands-on-time. Automated technologies are restricted in number of proteins measured, decreasing the ability to evaluate the full spectrum of cytokine profiles.

The CodePlex Secretome platform addresses this gap by providing highly multiplexed bulk analysis with a completely automated workflow that reduces the risk for user variability and error while increasing hands-off, walkaway time. CodePlex chips have been validated to work with a variety of sample types, including cell culture supernatant, cell lysate, plasma, serum, cerebrospinal fluid, lung lavage/tracheal wash, and urine, providing a flexible multiplexed analysis platform which requires just 11 µL of sample volume (5.5 µL per

replicate). CodePlex chips measure up to 22 cytokines in bulk, automated on the Bruker system, and can selectively run eight conditions per chip in “MacroChambers” across eight chips on a single run. CodePlex offers a modular solution to analyze as few as eight small volume samples at a time, up to 64 samples, instead of requiring a full 96 samples before generating multiplexed bulk cytokine data. The CodePlex Secretome platform is uniquely measuring multiplexed bulk cytokine metrics in an automated manner[11-13].

Samples that have been prepared according to validated protocols are loaded into individual chambers on the CodePlex chip which is then inserted into the instrument. Proteomic analysis occurs using an automated sandwich ELISA method, where capture antibodies are bound to a glass slide in a specific spatial arrangement covering the sample chambers. This antibody “barcode” allows for the highly multiplexed identification of secreted functional proteins within each chamber, resulting in characterization across all chambers in a parallelized manner. Imaging occurs after the automated ELISA protocol is complete and data related to intensity and spatial location of fluorophores are exported to the IsoSpeak software for analysis.

Bruker Instruments

Bruker instruments, the IsoLight, IsoSpark, and IsoSpark Duo are the only systems that enable researchers to get the highly multiplexed cytokine data with no advanced training, and no interaction with the samples. Furthermore, **Bruker Instruments are the only systems to perform multiplexed proteomic detection of 30+ cytokine markers simultaneously, to provide early predictive metrics of functional proteins,** in an automated, all-in-one system, with increased work-away time. This system can handle a smaller amount of sample volume if large blood draws are not possible, making it capable of handling a wider range of clinical sample sizes.

Featured Application	High-Capacity Instrument	Personal Lab Instrument	Complete Immune Landscaping
Walk-Away Proteomics Workflow	●	●	●
Immediate Predictive Insights	●	●	●
Publication-Ready Visualizations	●	●	●
Compatible with All Applications	●	●	●
Run Multiple Applications at Once			●
Chips Throughput	8 Chips	4 Chips	8 Chips
Instrument Footprint	28.5 in	18 in	36 in

1. IsoLight

The IsoLight is a hub for comprehensive, high throughput proteomic profiling of various cell types across a large assay menu of single-cell and population chip and software products. With one instrument, achieve functional immune profiling at the single-cell level, analyze intracellular signaling omics, and run high-plex walkaway immunoassays. Through IsoCode Chips, Single-Cell Secretome and Single-Cell Signaling, the IsoLight captures single-cell, functional proteomics profiles from thousands of single cells in parallel to better understand complex immunotherapy patient responses. Through CodePlex Chips, the IsoLight enables highly multiplexed proteomics from low sample volumes to generate bulk cytokine data via a fully automated workflow. Our IsoSpeak bioinformatics platform helps discover new patient relationships among heterogeneous cells and clearly defines subsets of powerful, polyfunctional cells, secreting 2+ cytokines simultaneously, that can help predict patient outcomes and determine disease progression. IsoLight has won The Scientist's Top 10 Innovations 2017 award, The Edison Award 2019, and has been nominated for Red Dot Product Design award 2018.

2. IsoSpark

The IsoSpark is a personalized functional proteomics system for every lab. The intuitive system fits on any lab bench and accelerates personalized medicine across research areas & high-impact applications. Across all applications, discover direct functional profiling of single-cell and bulk population insights with walk-away automation. Through IsoCode Chips, Single-Cell Secretome and Single-Cell Signaling, the IsoSpark captures single-cell, functional proteomics profiles from thousands of single cells in parallel to better understand complex immunotherapy patient responses. Through CodePlex Chips, the IsoSpark enables highly multiplexed proteomics from low sample volumes to generate bulk cytokine data via a fully automated workflow. Our IsoSpeak bioinformatics platform helps discover new patient relationships among heterogeneous cells and clearly defines subsets of powerful, polyfunctional cells, secreting 2+ cytokines simultaneously, that can help predict patient outcomes and determine disease progression.

3. IsoSpark Duo

The IsoSpark Duo platform, which runs the IsoCode and CodePlex Chips, is a versatile, precision automation platform designed to understand and characterize differences among immune cells, mapping thousands of cells per sample to reveal complete functional profiles and polyfunctionality among cell subsets. The advanced setup enables complete functional immune landscaping through the IsoSpark Duo's ability to run multiple applications at one. Through IsoCode Chips, Single-Cell Secretome and Single-Cell Signaling, the IsoSpark Duo captures single-cell, functional proteomics profiles from thousands of single cells in parallel to better understand complex immunotherapy patient response. Through CodePlex Chips, the IsoSpark Duo enables highly multiplexed proteomics from low sample volumes to generate bulk cytokine data via a fully automated workflow. Our IsoSpeak bioinformatics platform helps discover new patient relationships among heterogeneous cells and clearly defines subsets of powerful, polyfunctional cells, secreting 2+ cytokines simultaneously, that can help predict patient outcomes and determine disease progression.

IsoSpeak

The integrated IsoSpeak data informatics software provides same-day turnaround of publication-ready data visualizations to stratify samples, reveal functional differences, pinpoint biological drivers, and allow better collaboration between research groups. IsoSpeak helps discover new patient relationships amongst heterogeneous cells, clearly defining subsets of powerful, multi-functional cells that can help predict patient outcome and determine disease progression. The intuitive user interface allows for push-button visualizations, eliminating the need for specialized personnel, extensive training, and weeks or months of waiting for critical data insights.

The IsoSpeak software is included with all instruments and is available for download by customers. Once raw data is imported into IsoSpeak and experiment parameters have been identified, the data processing capability assesses background levels for each analyte being measured and gates signals using validated methods, ensuring that generated signals are reliable. Secondary analyses include signal intensity, signal distribution, polyfunctionality, PCA, UMAP, 2D and 3D t-SNE, replicate correlation, and protein correlation, depending on the application. The push-button interface with integrated analyses allows for the rapid data analysis and generation of interactive visualizations for data exploration.

Polyfunctional Overview

3D Cytokine Mapping tSNE

Polyfunctional Strength Index

Polyfunctional Heat Map

Applications

Response

Friedman CF, Spencer C, Cabanski CR, et al. Ipilimumab alone or in combination with nivolumab in patients with advanced melanoma who have progressed or relapsed on PD-1 blockade: clinical outcomes and translational biomarker analyses. *J Immunother Cancer*. 2022;10(1):e003853. doi:10.1136/jitc-2021-003853

Lee JB, Khan DH, Hurren R, et al. Venetoclax enhances T cell-mediated antileukemic activity by increasing ROS production. *Blood*. 2021;138(3):234-245. doi:10.1182/blood.2020009081

Diab A, Tykodi SS, Daniels GA, et al. Bempegaldesleukin Plus Nivolumab in First-Line Metastatic Melanoma. *J Clin Oncol*. 2021;39(26):2914-2925. doi:10.1200/JCO.21.00675

Rossi J, Paczkowski P, Shen YW, et al. Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. *Blood*. 2018;132(8):804-814. doi:10.1182/blood-2018-01-828343

Abbas HA, Alaniz Z, Mackay S, et al. Single-cell polyfunctional proteomics of CD4 cells from patients with AML predicts responses to anti–PD-1–based therapy. *Blood Adv*. 2021;5(22):4569-4574. doi:10.1182/bloodadvances.2021004583

Zhou J, Kaiser A, Ng C, et al. CD8+ T-cell mediated anti-malaria protection induced by malaria vaccines; assessment of hepatic CD8+ T cells by SCBC assay. *Hum Vaccin Immunother*. 2017;13(7):1625-1629. doi:10.1080/21645515.2017.1304333

Potency

Creelan BC, Wang C, Teer JK, et al. Tumor-infiltrating lymphocyte treatment for anti-PD-1-resistant metastatic lung cancer: a phase 1 trial. *Nat Med*. 2021;27(8):1410-1418. doi:10.1038/s41591-021-01462-y

Parisi G, Saco JD, Salazar FB, et al. Persistence of adoptively transferred T cells with a kinetically engineered IL-2 receptor agonist. *Nat Commun*. 2020;11(1):660. doi:10.1038/s41467-019-12901-3

Roselli E, Boucher JC, Li G, et al. 4-1BB and optimized CD28 co-stimulation enhances function of human mono-specific and bi-specific third-generation CAR T cells. *J Immunother Cancer*. 2021;9(10):e003354. doi:10.1136/jitc-2021-003354

Spiegel JY, Patel S, Muffly L, et al. CAR T cells with dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell malignancies: a phase 1 trial. *Nat Med*. 2021;27(8):1419-1431. doi:10.1038/s41591-021-01436-0

Li D, Li N, Zhang YF, et al. Persistent Polyfunctional Chimeric Antigen Receptor T Cells That Target Glypican 3 Eliminate Orthotopic Hepatocellular Carcinomas in Mice. *Gastroenterology*. 2020;158(8):2250-2265.e20. doi:10.1053/j.gastro.2020.02.011

Zhu H, Blum RH, Bernareggi D, et al. Metabolic Reprograming via Deletion of CISH in Human iPSC-Derived NK Cells Promotes In Vivo Persistence and Enhances Anti-tumor Activity. *Cell Stem Cell*. 2020;27(2):224-237.e6. doi:10.1016/j.stem.2020.05.008

Huang J, Zhou J, Ghinnagow R, et al. Targeted Co-delivery of Tumor Antigen and α-Galactosylceramide to CD141+ Dendritic Cells Induces a Potent Tumor Antigen-Specific Human CD8+ T Cell Response in Human Immune System Mice. Front Immunol. 2020;11:2043. Published 2020 Aug 18. doi:10.3389/fimmu.2020.02043

Persistence

Parisi G, Saco JD, Salazar FB, et al. Persistence of adoptively transferred T cells with a kinetically engineered IL-2 receptor agonist. *Nat Commun*. 2020;11(1):660. doi:10.1038/s41467-019-12901-3

Roselli E, Boucher JC, Li G, et al. 4-1BB and optimized CD28 co-stimulation enhances function of human mono-specific and bi-specific third-generation CAR T cells. *J Immunother Cancer*. 2021;9(10):e003354. doi:10.1136/jitc-2021-003354

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Survival

Diab A, Tykodi SS, Daniels GA, et al. Bempegaldesleukin Plus Nivolumab in First-Line Metastatic Melanoma. *J Clin Oncol*. 2021;39(26):2914-2925. doi:10.1200/JCO.21.00675

Abbas HA, Alaniz Z, Mackay S, et al. Single-cell polyfunctional proteomics of CD4 cells from patients with AML predicts responses to anti–PD-1–based therapy. *Blood Adv*. 2021;5(22):4569-4574. doi:10.1182/bloodadvances.2021004583

Fitness

Lee JB, Khan DH, Hurren R, et al. Venetoclax enhances T cell-mediated antileukemic activity by increasing ROS production. *Blood*. 2021;138(3):234-245. doi:10.1182/blood.2020009081

Inflammation

Su Y, Yuan D, Chen DG, et al. Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell*. 2022;185(5):881-895.e20. doi:10.1016/j.cell.2022.01.014

Szabo PA, Dogra P, Gray JI, et al. Longitudinal profiling of respiratory and systemic immune responses reveals myeloid cell-driven lung inflammation in severe COVID-19. *Immunity*. 2021;54(4):797-814.e6. doi:10.1016/j.immuni.2021.03.005

Farhadian S, Glick LR, Vogels CBF, et al. Acute encephalopathy with elevated CSF inflammatory markers as the initial presentation of COVID-19. *Res Sq*. Published online May 12, 2020:rs.3.rs-28583. doi:10.21203/rs.3.rs-28583/v1

Su Y, Chen D, Yuan D, et al. Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19. Cell. 2020;183(6):1479-1495.e20. doi:10.1016/j.cell.2020.10.037

Allorejection

Xie CB, Zhou J, Mackay S, Pober JS. Complement-activated human endothelial cells stimulate increased polyfunctionality in alloreactive T cells. *Am J Transplant*. 2021;21(5):1902-1909. doi:10.1111/ajt.16485

Pathogenesis

Chen YP, Kim HJ, Wu H. SIRPα expression delineates subsets of intratumoral monocyte/macrophages with different functional and prognostic impact in follicular lymphoma. *Blood Cancer J*. 2019;9:84. doi:10.1038/s41408-019-0246-0

Cytokine Release Syndrome (CRS)

Rossi J, Paczkowski P, Shen YW, et al. Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. *Blood*. 2018;132(8):804-814. doi:10.1182/blood-2018-01-828343

Resistance

Wei W, Shin YS, Xue M, et al. Single-Cell Phosphoproteomics Resolves Adaptive Signaling Dynamics and Informs Targeted Combination Therapy in Glioblastoma. *Cancer Cell*. 2016;29(4):563-573. doi:10.1016/j.ccell.2016.03.012

Su Y, Ko ME, Cheng H, et al. Multi-omic single-cell snapshots reveal multiple independent trajectories to drug tolerance in a melanoma cell line. *Nat Commun*. 2020;11(1):2345. doi:10.1038/s41467-020-15956-9

Su Y, Chen D, Yuan D, et al. Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19. *Cell*. 2020;183(6):1479-1495.e20. doi:10.1016/j.cell.2020.10.037

Bruker Panel Menu

**Note: Inquire about availability*
***Note: In pipeline*

Chip Type	Description	Available Panels	Analytes
Single-Cell Secretome	Automated analysis of single-cell secreted cytokines	Human Adaptive Immune	Granzyme B, IFN-γ, MIP-1α, Perforin, TNF-α, TNF-β, GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21*, CCL11, IP-10, MIP-1β, RANTES, IL-4, IL-10, IL-13, IL-22, TGFβ1, sCD137, sCD40L, IL-1β, IL-6, IL-17A, IL-17F, MCP-1, MCP-4
		Non-Human Primate Adaptive Immune	TNF-α, MCP-1, IL-2, IL-4, MIP-1β, IL-6, IL-8, IL-1β, RANTES, IFN-g, IP-10, MIP-1α, MIF, GM-CS
		Mouse Adaptive Immune	Granzyme B, IFN-γ, MIP-1α, TNF-α, GM-CSF, IL-2, IL-5, IL-7, IL-12p70, IL-15, IL-18, IL-21, sCD137, CCL11, CXCL1, CXCL13, IP-10, RANTES, Fas, IL-4, IL-10, IL-13, IL-27, TGFβ1, IL-6, IL-17A, MCP-1, IL-1β
		Human Innate Immune	IFN-γ, MIP-1α, TNF-α, TNF-β, GM-CSF, IL-8, IL-9, IL-15, IL-18, TGF-α, IL-5, CCL11, IP-10, MIP-1β, RANTES, BCA-1, IL-10, IL-13, IL-22, sCD40L, IL-1β, IL-6, IL-12-p40, IL-12, IL-17A, IL-17F, MCP-1, MCP-4, MIF, EGF, PDGF-BB
		Human Inflammation	GM-CSF, IFN-γ, IL-2, IL-12, TNF-α, TNF-β, IL-4, IL-5, IL-7, IL-9, IL-13, CCL11, IL-8, IP-10, MCP-1, MCP-4, MIP-1α, MIP-1β, RANTES, IL-10, IL-15, IL-22, TGF-β1, IL-1β, IL-6, IL-17A, IL-17F, IL-21*, Granzyme B, Perforin, sCD40L, sCD13
		Mouse Innate Immune	IFN-g, TNF-a, MIP-1a, IL-15, GM-CSF, IL-5, IL-10, IL-13, IL-6, IL-17A, MCP-1, IP-10, MIP-1b, EGF, PDGF-BB, MIF
		Human Natural Killer	Granzyme B, IFN-γ, MIP-1α, Perforin, TNF-α, TNF-β, GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21*, CCL11, IP-10, MIP-1β, RANTES, IL-4, IL-10, IL-13, IL-22, TGFβ1, sCD137, sCD40L, IL-1β, IL-6, IL-17A, IL-17F, MCP-1, MCP-4
Single-Cell Signaling	Automated analysis of single-cell intracellular proteins	Human Tumor Signaling	P-PRAS40, P-IkBα, P-NF-kβ p65, P-Met, P-p44/42 MAPK, P-S6 Ribosomal, P-p90RSK, P-STAT3, P-MEK1/2, P-Stat1, P-Stat5, P-eIF4E, Cleaved PARP*, Alpha Tubulin
		Adaptive Signaling**	P-MEK1/2, P-NF-κB p65, P-Stat1, P-Stat3, P-Stat5, Granzyme B, IFN-γ, Perforin, TNF-α, GM-CSF, IL-2, IL-7, IL-8, IL-10, MIP-1a, MIP-1B
		Myeloid Signaling**	P-MEK1/2, P-NF-κB p65, P-Stat1, P-Stat3, P-Stat5, GM-CSF, IL-8, IL-10, TNF-a, IL-1B, IL-6, MIP-1a, MIP-1B, MCP-1
CodePlex Secretome	Highly Multiplexed Bulk Immunoassay	Human Adaptive Immune	GM-CSF, Granzyme B, IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, IL-15, IL-17A, IP-10, MCP-1, MIP-1α, MIP-1β, Perforin, sCD137, TNF-α, TNF-β
		Human Innate Immune	EGF, GM-CSF, Granzyme B, IFN-γ, IL-1β, IL-4, IL-6, IL-7, IL-8, IL-10, IL-15, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-BB, sCD137, TNF-α, VEGF
		Human Cytokine Storm	GM-CSF, IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, IL-17A, IP-10, MCP-1, MIP-1α, MIP-1β, Perforin, TNF-α
		Human Stem Cell Signaling	IL-17A, MIP-1α, IL-6, IL-4, MIP-1β, IL-8, IFN-γ, GM-CSF, IL-10, TNF-α, MCP-1, IL-2, IL-15, RANTES, IL-1α, IL-1β, CXCL5
		Human Cancer Signaling	EGF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, MCP-1, MIF, PDGF-BB, RANTES, TNF-α
		Mouse Adaptive Immune	GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-17A, IP-10, KC, MCP-1, MIP-1α, RANTES, TNF-α
		Mouse Inflammation	IFN-γ, TNF-α, MIP-1α, IL-2, IL-5, IL-10, IL-13, IL-4, IL-6, IL-1β, IL-17A, IL-12, MCP-1, IP-10, KC, GM-CSF
		Mouse Innate Immune	IFN-γ, TNF-α, MIP-1α, IL-15, GM-CSF, IL-5, IL-10, IL-13, IL-6, IL-17A, MCP-1, IP-10, MIP-1β, EGF, PDGF-BB, MIF
		Non-Human Primate Adaptive Immune	GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, TNF-α
		Human Tumor Signaling**	P-PRAS40, P-IkBα, P-NF-kβ p65, P-Met, P-p44/42 MAPK, P-S6 Ribosomal, P-p90RSK, P-STAT3, P-MEK1/2, P-Stat1, P-Stat5, P-eIF4E, Cleaved PARP, Alpha Tubulin
		Mouse Stem Cell Signaling**	GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-15, IL-17A, MCP-1, MIP-1α, MIP-1β, RANTES, TNF-α
		Mouse Cancer Signaling**	EGF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-13, MCP-1, MIF, PDGF-BB, RANTES, TNF-α, VEGF

Instrument Specification Sheets

IsoLight System Specifications	IsoLight Performance Specifications
Working Environment • For indoor use only • Operating temperature: 65 – 77°F (18 – 25°C) • Humidity: 40 – 60%, non-condensing • Altitude: < 6,500 ft (2,000 m) **Dimensions** • Width: 28.5 in (72.5 cm) • Height: 30.5 in (77.5 cm) • Depth: 28 in (71 cm) **Weight** • Crated for shipping: 338 lb (154 kg) • Free standing: 255 lb (116 kg) **Bench Size** • Width: > 36 in (91 cm) • Depth: > 24 in (61 cm) **Clearance** • Front: > 36 in (91 cm) aisle for operator access • Rear: > 4 in (10 cm) • Left: > 4 in (10 cm) • Right: > 4 in (10 cm) **Power Supply** • Voltage: 100 V (min) to 240 V (max) • Current: 6.3 A (max) • Frequency: 50/60 Hz **Gas Supply** • Connection: 0.25 in or 4 mm OD push to connect tubing • Pressure: 30-70 PSI • Composition: Carbon dioxide (CO_2) at > 99% purity **User Interface** • 24 in IPS 10-point multi-touch screen • RGB LED status indicator **Connection** • Ethernet: 1xGigE • USB: 2x USB 3.0	**Consumables** • Up to 8 disposable IsoCode® Chips per run with barcode tracking **Reagents** • Disposable one-time use reagents **Cell Counts** • > 1,000 isolated single cells per chip • > 8,000 isolated cells per run with 8 chips **Throughput** • Over 30-plex functional cytokines per isolated single cell • Over 300,000 single cell, secreted protein data points per run **Hands-On Time** • < 3 min per sample (cell preparation time not included) **Run Time** • < 24 hours from sample loading to results **On-Board Incubator** • Temperature: 37 ± 2°C • CO_2 concentration: 5 ± 1% **Laser Wavelengths** • 405 nm, 473 nm, 638 nm **Software Solutions** • IsoSpeak® data analysis software

Bruker Grant Support Package

IsoSpark System Specifications

Working Environment
- For indoor use only
- Operating temperature: +15°C ~ +30°C (59°F ~ 86°F)
- Humidity: 20% ~ 80%, non-condensing
- Altitude: < 6,500 ft (2,000 m)

Dimensions
- Width: 18 in (45.7 cm)
- Height: 19.8 in (50.3 cm)
- Depth: 19.7 in (50.0 cm)

Weight
- Crated for shipping: 140 lb (63.5 kg)
- Free standing: 95.5 lb (43.4 kg)

Bench Size
- Width: > 30 in (76 cm)
- Depth: > 23.7 in (60.2 cm)

Clearance
- Front: > 4 in (10 cm)
- Rear: > 4 in (10 cm)
- Left: > 12 in (30 cm)
- Right: > 12 in (30 cm)
- Height: > 12 in (30 cm)

Power Supply
- Voltage: 100 V (min) to 240 V (max)
- Current: 6.3 A (max)
- Frequency: 50/60 Hz

Gas Supply
- Connection: 0.25 in or 4 mm OD push to connect tubing
- Pressure: 30-70 PSI
- Composition: Carbon dioxide (CO_2) at > 99% purity

User Interface
- 11 in LCD multi-touch screen

Connection
- Ethernet: 1xGigE
- USB: 3x USB 3.0, 2 front & 1 rear cable

IsoSpark Performance Specifications

Consumables
- Up to 4 disposable IsoCode® or CodePlex®
- Chips per run with barcode tracking

Reagents
- Disposable one-time use reagents

Cell Counts
- 500-1500 targeted single cells per chip
- 2000-6000 targeted cells per run with 4 chips

Throughput
- Over 30-plex functional cytokines per isolated single cell
- Over 150,000 single cell, secreted protein data points per run

Hands-On Time
- < 3 min per sample (cell preparation time not included)

Run Time
- < 24 hours from sample loading to results

On-Board Incubator
- Temperature: 37 ± 2°C
- CO_2 concentration: 5 ± 1%

Laser
- Wavelengths: 405 nm, 473 nm, 638 nm
- Safety: class 1 laser product

Software Solutions
- IsoSpeak® data analysis software
- Operating System: PC

Additional Resources

For more information about how Bruker's platforms can advance your research, explore these helpful resources:

- Bruker | The Functional Cell Biology Company
- Publications & Presentations
- Product Literature
- Product & Workflow Support
- Functional Cell Library

References

1. Lu Y, Chen JJ, Mu L, Xue Q, Wu Y, Wu PH, Li J, Vortmeyer AO, Miller-Jensen K, Wirtz D, Fan R. High-throughput secretomic analysis of single cells to assess functional cellular heterogeneity. Anal Chem. 2013 Feb 19;85(4):2548-56. doi: 10.1021/ac400082e. Epub 2013 Feb 1. PMID: 23339603; PMCID: PMC3589817.
2. Riou, C., Burgers, W.A., Mlisana, K., Koup, R.A., Roederer, M., Abdool Karim, S.S., Williamson, C. & Gray, C.M. Differential impact of magnitude, polyfunctional capacity, and specificity of HIV-specific CD8+ T cell responses on HIV set point. *J Virol* **88**, 1819-1824 (2014). 3911608
3. Duvall, M.G., Precopio, M.L., Ambrozak, D.A., Jaye, A., McMichael, A.J., Whittle, H.C., Roederer, M., Rowland-Jones, S.L. & Koup, R.A. Polyfunctional T cell responses are a hallmark of HIV-2 infection. *Eur J Immunol* **38**, 350-363 (2008). 2362391
4. Ma, C., Fan, R., Ahmad, H., Shi, Q.H., Comin-Anduix, B., Chodon, T., Koya, R.C., Liu, C.C., Kwong, G.A., Radu, C.G., Ribas, A. & Heath, J.R. A clinical microchip for evaluation of single immune cells reveals high functional heterogeneity in phenotypically similar T cells. *Nature Medicine* **17**, 738-U133 (2011).
5. Ma, C., Cheung, A.F., Chodon, T., Koya, R.C., Wu, Z., Ng, C., Avramis, E., Cochran, A.J., Witte, O.N., Baltimore, D., Chmielowski, B., Economou, J.S., Comin-Anduix, B., Ribas, A. & Heath, J.R. Multifunctional T-cell analyses to study response and progression in adoptive cell transfer immunotherapy. *Cancer Discov* **3**, 418-429 (2013). PMC3716460
6. Friedman CF, Spencer C, Cabanski CR, et al. Ipilimumab alone or in combination with nivolumab in patients with advanced melanoma who have progressed or relapsed on PD-1 blockade: clinical outcomes and translational biomarker analyses. J Immunother Cancer. 2022;10(1):e003853. doi:10.1136/jitc-2021-003853
7. Abbas HA, Alaniz Z, Mackay S, et al. Single-cell polyfunctional proteomics of CD4 cells from patients with AML predicts responses to anti-PD-1-based therapy. Blood Adv. 2021;5(22):4569-4574. doi:10.1182/bloodadvances.2021004583

8. Wei, W., Shin, Y. S., Xue, M., Matsutani, T., Masui, K., Yang, H., Ikegami, S., Gu, Y., Herrmann, K., Johnson, D., Ding, X., Hwang, K., Kim, J., Zhou, J., Su, Y., Li, X., Bonetti, B., Chopra, R., James, C. D., Cavenee, W. K., … Gini, B. (2016). Single-Cell Phosphoproteomics Resolves Adaptive Signaling Dynamics and Informs Targeted Combination Therapy in Glioblastoma. Cancer cell, 29(4), 563–573. https://doi.org/10.1016/j.ccell.2016.03.012
9. Su, Y., Ko, M. E., Cheng, H., Zhu, R., Xue, M., Wang, J., Lee, J. W., Frankiw, L., Xu, A., Wong, S., Robert, L., Takata, K., Yuan, D., Lu, Y., Huang, S., Ribas, A., Levine, R., Nolan, G. P., Wei, W., Plevritis, S. K., … Heath, J. R. (2020). Multi-omic single-cell snapshots reveal multiple independent trajectories to drug tolerance in a melanoma cell line. Nature communications, 11(1), 2345. https://doi.org/10.1038/s41467-020-15956-9
10. Su, Y., Wei, W., Robert, L., Xue, M., Tsoi, J., Garcia-Diaz, A., Homet Moreno, B., Kim, J., Ng, R. H., Lee, J. W., Koya, R. C., Comin-Anduix, B., Graeber, T. G., Ribas, A., & Heath, J. R. (2017). Single-cell analysis resolves the cell state transition and signaling dynamics associated with melanoma drug-induced resistance. Proceedings of the National Academy of Sciences of the United States of America, 114(52), 13679–13684. https://doi.org/10.1073/pnas.1712064115
11. Szabo, P. A., Dogra, P., Gray, J. I., Wells, S. B., Connors, T. J., Weisberg, S. P., Krupska, I., Matsumoto, R., Poon, M., Idzikowski, E., Morris, S. E., Pasin, C., Yates, A. J., Ku, A., Chait, M., Davis-Porada, J., Guo, X. V., Zhou, J., Steinle, M., Mackay, S., … Farber, D. L. (2021). Longitudinal profiling of respiratory and systemic immune responses reveals myeloid cell-driven lung inflammation in severe COVID-19. Immunity, 54(4), 797–814.e6. https://doi.org/10.1016/j.immuni.2021.03.005
12. Jayatilaka, H., Tyle, P., Chen, J. J., Kwak, M., Ju, J., Kim, H. J., Lee, J., Wu, P. H., Gilkes, D. M., Fan, R., & Wirtz, D. (2017). Synergistic IL-6 and IL-8 paracrine signalling pathway infers a strategy to inhibit tumour cell migration. Nature communications, 8, 15584. https://doi.org/10.1038/ncomms15584
13. Farhadian, S., Glick, L. R., Vogels, C., Thomas, J., Chiarella, J., Casanovas-Massana, A., Zhou, J., Odio, C., Vijayakumar, P., Geng, B., Fournier, J., Bermejo, S., Fauver, J. R., Alpert, T., Wyllie, A. L., Turcotte, C., Steinle, M., Paczkowski, P., Cruz, C. D., Wilen, C., … Aoun Barakat, L. (2020). Acute encephalopathy with elevated CSF inflammatory markers as the initial presentation of COVID-19. Research square, rs.3.rs-28583. https://doi.org/10.21203/rs.3.rs-28583/v1