APPLICATION HIGHLIGHT

Key Functional Insights Uncovered with CodePlex High-Plex Low Volume Immunoassays

A streamlined and efficient solution for highly multiplexed functional proteomics from very low sample volumes.

In this Application Note we outline:

- Why detecting multiplexed serum protein from ultra low sample volumes is critical in predicting responses in multiple research areas
- How conventional multiplexed bulk cytokine platforms compare to IsoPlexis platforms
- How IsoPlexis low sample volume and modular system allows for flexible sample numbers
- Identification of key inflammatory mediators from airway samples in severe COVID-19 patients
- Cytokine storm and neurological manifestations of COVID-19 in patients
- Understanding cancer cell communication to infer a strategy to inhibit tumor cell metastasis
- Accelerating regenerative medicine with fibroblasts
- Resolving heterogeneity in cytokine production profiles among HSPCs

Prep, Run, Analyze

CodePlex Secretome Panels

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

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

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 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 Panel
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 - Coming Soon
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 - Coming Soon
EGF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, MCP-1, MIF, PDGF-BB, RANTES, TNF-α

Status Quo Multiplexed Bulk Analysis
X Up to 100-200 uL per sample (for replicates)
X 6-10 hours of hands-on sample prep time
X Workflow requires multiple steps and user interaction points
X Fill 96 samples before run
X Multiple systems required to generate and analyze data
X Limit of Detection: 5-5000 pg/ml
X Data analysis and visualizations require much user input and are not automated

CodePlex Secretome
✓ 11 uL per samples (for replicates)
✓ 5 minutes of hands-on time
✓ Completely automated workflow
✓ Modular, load 8-64 samples per run
✓ One system: The IsoLight/IsoSpark
✓ Limit of Detection: 5-5000 pg/ml
✓ State-of-the-art data analysis software with advanced visualizations

CodePlex's high multiplexing capabilities enable targeting of up to 20+ cytokines. With extensive panel options and a fully hands-off workflow, CodePlex helps you maximize efficiency, providing key functional insights on one fully-integrated system.

Detecting Multiplexed Serum Protein from Ultra Low Sample Volume is Critical in Predicting Responses in Multiple Research Areas

The CodePlex family of solutions enables you to automate your entire workflow with one system, giving you the ability to run an entire highly multiplexed ELISA workflow in a completely automated and hands-off manner. Traditional multiplexed bulk ELISA platforms require multiple instruments and multiple personnel, in addition to long wait times for data analysis. With CodePlex, you can add your sample and walk away, achieving fully analyzed data on the same day. No additional equipment is needed. A fully automated workflow means no washing and incubation stations, no centrifuge, no vortexer, and no plate reader. You can unlock your data immediately, lowering costs per run and total instrumentation requirements. All of your proteomic needs are integrated into the completely automated IsoLight or IsoSpark system.

CodePlex chips measure up to 20+ cytokines in bulk, automated on the IsoPlexis 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.

Prep, Run, Analyze

Conventional Multiplexed Bulk Cytokine Platform vs IsoPlexis Benchtop Systems

CONVENTIONAL MULTIPLEXED BULK CYTOKINE PLATFORM

6-10 HOURS FOR USER

Step 1: Pre-wet plate
Step 2: Sample and reagents
Step 3: Shake
Step 4: Wash 2x
Step 5: Detection antibody
Step 6: Shake
Step 7: Wash 2x
Step 8: SA-Fluorophore
Step 9: Shake
Step 10: Wash 3x
Step 11: Wash Buffer
Step 12: Shake

DATA ANALYSIS

Step 13: Read in System
Step 14: Further analysis

ISOPLEXIS AUTOMATED BENCHTOP SYSTEM

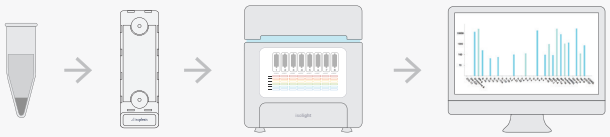
5 MINS FOR USER

Step 1: One-pipette-action per sample, no ELISA procedures by user

AUTOMATED ON ISOLIGHT

Step 2: All ELISA procedures performed automatically. Software delivers results.

ISOPLEXIS STEPS FOR FUNCTIONAL IMMUNE PROFILING

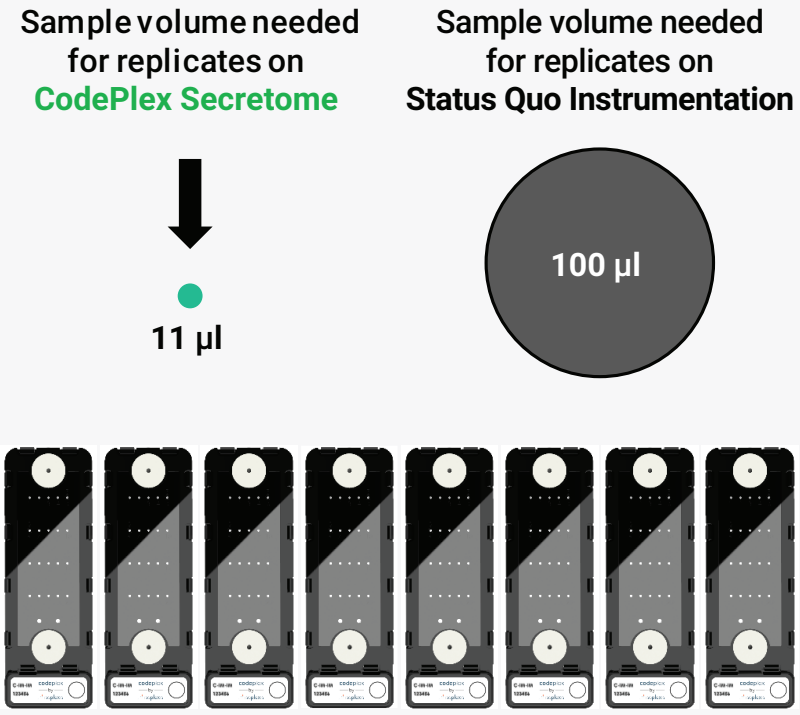


CodePlex requires only five minutes of hands-on time, compared to conventional multiplexed bulk cytokine ELISA platforms which require six to ten hours of hands on time for the user. All ELISA procedures are automated on the IsoLight or IsoSpark, and data is fully analyzed via IsoSpeak software.

- CodePlex requires only five minutes of hands on time versus six to ten hours of hands on time with a conventional multiplexed bulk cytokine platform.
- With a conventional platform, preparation requires multiple steps, as well as multiple instruments.
- With IsoPlexis systems, samples are simply loaded onto the CodePlex Secretome chip and loaded into the IsoLight or IsoSpark system.
- None of the ELISA procedures are performed by the user, as they are fully automated within the system. With the IsoSpeak software suite, data is instantly fully analyzed and visualized, saving both time and costs

Prep, Run, Analyze

IsoPlexis Requires 10x Less Sample Volume and Allows for Flexible Sample Numbers



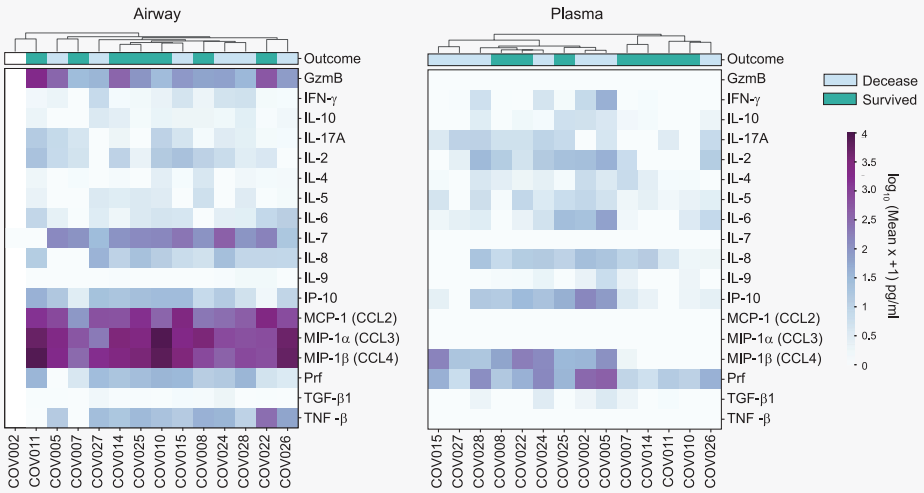
The CodePlex Secretome Solution measures up to 20+ cytokines in bulk, automated on the IsoLight system, and can selectively run eight conditions a chip in “MacroChambers” across eight chips on a single run. Easily run replicates with a small sample volume: (11 uL per sample replicate).

- CodePlex only requires a sample volume of 11µl for replicates in contrast to the 100µl or more sample volume needed on traditional technologies, allowing for the analysis of precious patient samples or in vivo mouse model samples.
- Codeplex also offers a modular solution to analyze as few as eight small volume samples at a time and up to 64 samples, instead of requiring a full 96 samples before generating multiplexed bulk cytokine data.

Application 1 – Highly Multiplexed Bulk Proteomics Identifies Elevated Inflammatory Mediators in Airway Samples of Severe COVID-19

Analysis of Dynamic Immune Processes Fills Gaps in Understanding of Severe COVID-19 Pathogenesis

IsoPlexis' CodePlex Solution Identifies Key Inflammatory Mediators from Airway Samples in Severe COVID-19



IsoPlexis' highly multiplexed CodePlex Secretome provides measurements in paired airway and plasma samples from COVID-19 patients and demonstrates MCP-1, MIP-1α, and MIP-1β, granzyme B, IL-7, and TNF-β were significantly increased in airways compared to blood.

Highlights of Findings of Elevated Inflammatory Mediators

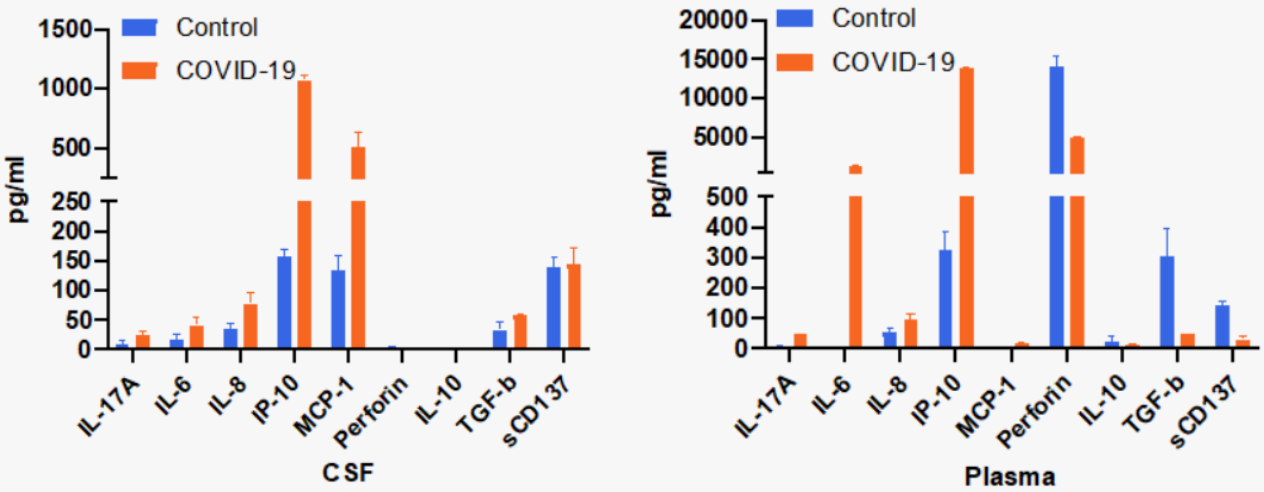
- CodePlex demonstrated markedly elevated monocyte/macrophage and T cell-derived cytokines in airways (tracheal lavage) of COVID-19 patients leading to inflammatory immune cells congregating in the lungs–particularly the monocyte chemoattractants MCP-1 (CCL2) and MIP-1α (CCL3).
- CodePlex was able to provide the qualitative and quantitative measurements in paired airway and plasma samples from COVID-19 patients and demonstrates MCP-1, MIP-1α, and MIP-1β, granzyme B, IL-7, and TNF-β were significantly increased in airways compared to blood.
- The detected excessive levels of MCP-1, MIP-1α and MIP-1β proteins in the airways but not in blood further support a role of airway monocytes/macrophages in perpetuating pulmonary inflammatory responses in severe COVID-19 and resulting in COVID-19-related acute respiratory distress syndrome (ARDS), which was associated with older age and mortality.
- IsoPlexis' CodePlex Secretome reveals hyper inflammatory immune responses predominantly in airways and provide additional evidence that targeting airway-derived cytokines such as CCL2 through CCR2 antagonists or other airway-specific mediators may be more effective in reducing lung damage or promoting recovery from ARDS in severe COVID-19.

P. Szabo et al., "Longitudinal profiling of respiratory and systemic immune responses reveals myeloid cell-driven lung inflammation in severe COVID-19," Immunity, 54(4):797-814.e6, 2021.

Application 2 – Cytokine Storm and Neurological Manifestations of COVID-19 in Patients

IsoPlexis' Functional Proteomics Reveals Secretomic Signatures in CSF and Plasma

Acute Encephalopathy with Elevated CSF Inflammatory Markers as the Initial Presentation of COVID-19



Secretion profiles of CSF and plasma in COVID-19 samples versus control.

Highlights of Findings of Unique Secretomic Signatures Revealed by CodePlex Secretome

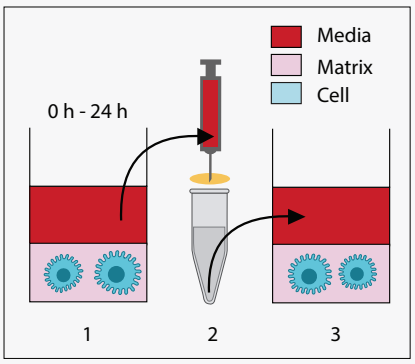
- CodePlex Secretome reveals unique cytokine signatures in plasma and CSF from patients presenting with neurological disorders in COVID-19.
- Findings suggest that neurologic symptoms such as encephalopathy and seizures may be the initial presentation of COVID-19.
- Central nervous system inflammation may associate with neurologic manifestations of disease.

Farhadian, S et al. Acute encephalopathy with elevated CSF inflammatory markers as the initial presentation of COVID-19. BMC Neurology 2020.

Application 3 – Understanding Cancer Cell Communication to Infer a Strategy to Inhibit Tumor Cell Metastasis

IsoPlexis' Functional Proteomics Reveals Unique Synergistic Paracrine Signaling Pathway Promoting Cell Migration

Secreted Cytokines Promote Tumor Metastasis



HT1080WT	10	20	50	100	120	150	R^2
EGF							0.07
FGF							0.01
HGF							0.01
VEGF							0.40
PDGF							0.02
MIF							0.82
MCP1							0.09
RANTES							0.57
MIP1a							0.01
IFNg							0.17
TNFa							0.22
TNFb							0.68
GMCSF							0.01
IL1a							0.17
IL1b							0.10
IL2							0.09
IL4							0.14
IL5							0.34
IL6							0.85
IL8							0.83
IL10							0.00
IL12							0.03
IL13							0.25
FITC							0.41

0 — 57.7AU

MDA-MB-231	10	20	50	100	R^2
EGF					0.91
FGF					0.86
HGF					0.03
VEGF					0.53
PDGF					0.06
MIF					0.27
MCP1					0.11
RANTES					0.00
MIP1a					0.00
IFNg					0.00
TNFa					0.38
TNFb					0.93
GMCSF					0.60
IL1a					0.11
IL1b					0.02
IL2					0.39
IL4					0.21
IL5					0.61
IL6					0.99
IL8					0.82
IL10					0.00
IL12					0.30
IL13					0.40
FITC					0.24

0 — 8.9AU

The secretome mediated pathway of cell migration is an adaptive process dictated by cell signaling. Understanding this mechanism provides a potential strategy towards decreasing the metastatic capacity of cancer cells.

Highlights of IsoPlexis' Functional Proteomics and Secretomic Signatures in Tumor Cell Migration

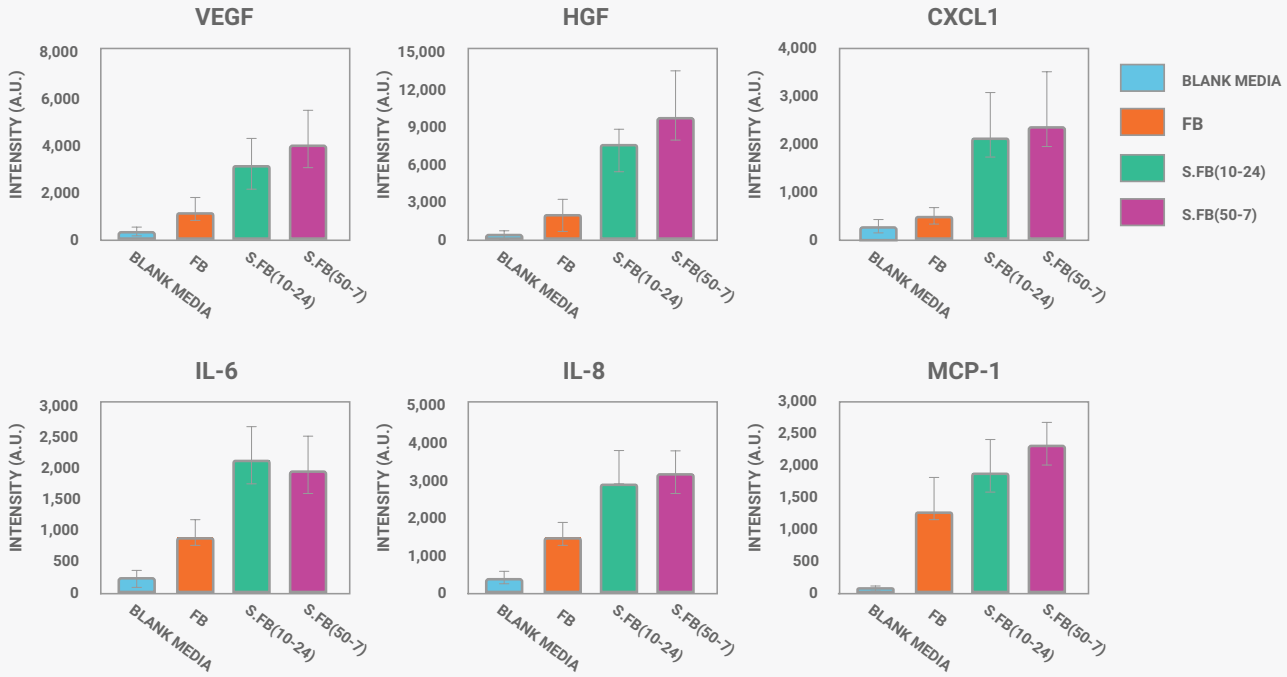
- IsoPlexis technology revealed IL-6 and IL-8 were secreted at high concentrations in a specific ratio and density-dependent manner.
- Proteins typically associated with promoting tumor metastasis and progression were not elevated, suggesting that IL-6 and IL-8 are responsible for driving the density-dependent cell migration within 3D matrices.
- The data reveals a possible mechanism for the promotion of tumor cell migration while inferring an approach to reduce metastatic capability of tumor cells.

Jayatilaka H, et al. Synergistic IL-6 and IL-8 Paracrine Signalling Pathway Infers a Strategy to Inhibit Tumour Cell Migration. Nature Communications, 8, 15584, 2017

Application 4 – Accelerating Regenerative Medicine with Fibroblasts

CodePlex Proteomics Reveals Insight into Fibroblast Driven Cellular Senescence

Uncovering the Secretome of Senescent Fibroblasts with CodePlex Secretome



Secreted factors from senescent fibroblasts that contribute to cellular regeneration.

Applying CodePlex Secretome to Cellular Senescence

- Factors from senescent fibroblasts contribute to inflammation and promotion of cancer development.
- Functional proteomics reveals insight into various challenges associated with delivering growth factors and cytokines for various therapeutic applications.
- Growth factor production from senescent fibroblasts was significantly increased compared to the presenescent fibroblasts, which demonstrates potential for promoting microvasculature formation *in vitro* and *in vivo*.

Xiao Y, et al. Senescent Cells with Augmented Cytokine Production for Microvascular Bioengineering and Tissue Repairs. Advanced Biosystems 3: 1Z900089, 2019.

Application 5 – Resolving Heterogeneity in Cytokine Production Profile Among HSPCs

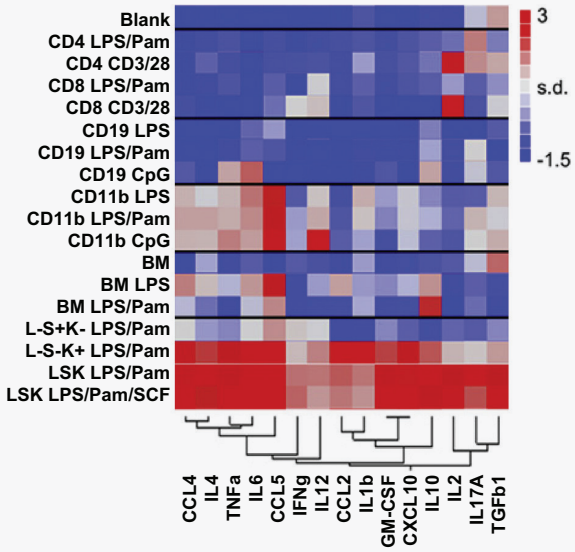
IsoPlexis' Functional Proteomics Reveals Secretomic Signatures in Stem and Progenitor Cells

Highlights of IsoPlexis' Functional Proteomics and Functional Significance of HSPC-Produced Cytokines

- CodePlex technology showed that in response to stimulation, T, B, and myeloid cells were still significantly less potent cytokine producers than LSK cells.
- This study has uncovered an important property of HSPCs that enables them to convert danger signals into versatile cytokine signals for the regulation of stress hematopoiesis.

Zhao JL, et al. Conversion of Danger Signals into Cytokine Signals by Hematopoietic Stem and Progenitor Cells for Regulation of Stress-Induced Hematopoiesis. Cell Stem Cell 14: 445-459, 2014.

Highly-Multiplexed Bulk Analysis of Stem and Progenitor Cell Types in Response to Infectious Pathogens



Highly-multiplexed proteomic quantification of cytokines in bulk cell-culture medium in order to compare cytokine production of different cell subsets.