TECHNICAL NOTE

Automate Your Entire Highly Multiplexed ELISA Workflow with CodePlex Secretome

Run an entire highly multiplexed ELISA workflow in a completely automated manner with CodePlex Secretome for the IsoLight system.

In this Technical Note we outline:

- The breakthrough CodePlex automated highly multiplexed proteomics workflow
- How Bruker's CodePlex Secretome solution is uniquely capturing key measures of highly multiplexed bulk cytokine metrics in an automated manner
- How a small sample volume (11ul) can be used to run replicates with the CodePlex Secretome solution versus status quo instrumentation (100ul)
- Next-generation improvements over conventional multiplexed ELISA workflows
- Understanding tumor metastasis with CodePlex Secretome
- Democratizing cellular proteomics to accelerate immune medicine

BRUKER

Automation of a Complete Highly Multiplexed Bulk ELISA Cytokine Assay

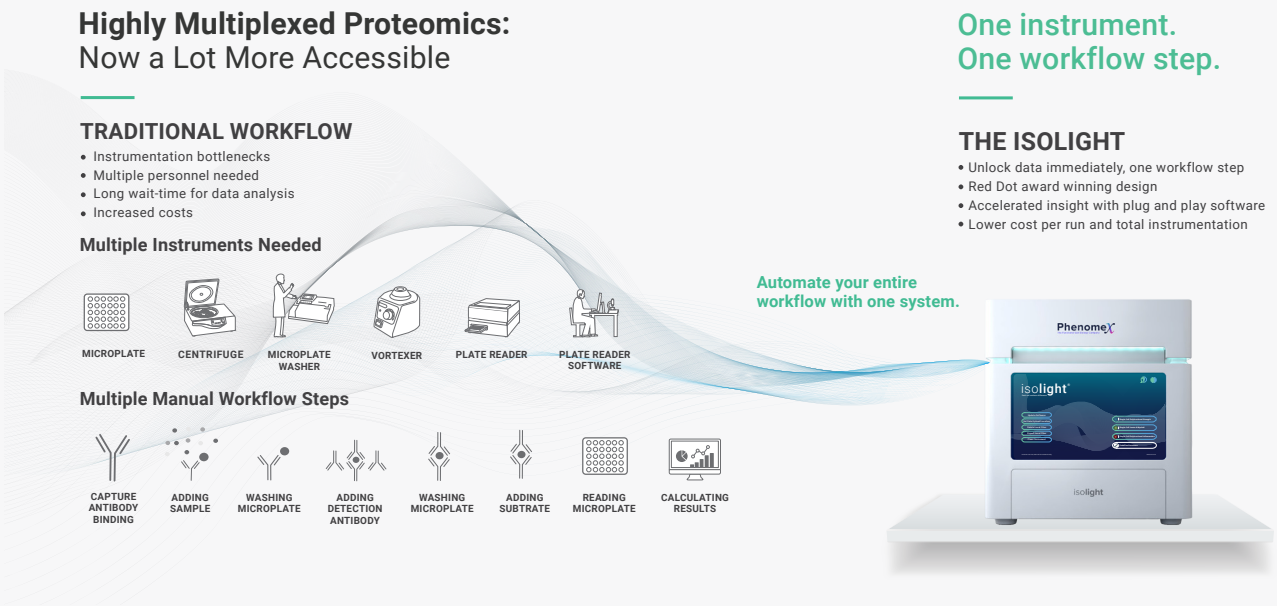

Figure 1 | Load up to 8 CodePlex chips onto the IsoLight system for a completely automated and hands-off end-to-end highly multiplexed proteomics workflow. All cellular analysis and washing steps associated with the ELISA assay are automated, minimizing the risk of human error. With advanced fluidics and precision imaging, samples are detected and analyzed automatically, helping you get answers faster.

Automated highly multiplexed proteomics workflow

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 (Figure 1). 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 system.

Bruker's CodePlex solution is uniquely capturing key measures of highly multiplexed bulk cytokine metrics in an automated manner

The mechanism for both clinical effects and cytotoxicity can be heavily mediated by cytokines (functional proteins through which immune cells send and receive signals). Thus, the ability to capture the range of relevant cytokines, fully automated in a highly multiplexed manner, is critical in obtaining the necessary data to accelerate your programs.

Bruker's CodePlex solution is helping researchers by providing a faster and more streamlined approach to generating highly multiplexed bulk cytokine data via a fully automated end-to-end workflow. It does this by offering a modular solution to bulk cytokine data analysis and minimizes variability from user input.

CodePlex Secretome chips are used to optimize experimental conditions by enabling quick and cost-efficient population assays. CodePlex chips measure 30+ cytokines in bulk (Figure 2a), automated on the IsoLight 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 (Figure 2b), up to 64 samples, instead of requiring a full 96 samples before generating multiplexed bulk cytokine data.

Improvements over conventional multiplexed ELISA workflow

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 (Figure 3). With a conventional platform, preparation entails adding reagents, adding detection antibodies, adding fluorophores, and incudes washing and shaking steps after each addition, as well as multiple instruments. With the IsoLight automated benchtop system, samples are simply loaded onto the CodePlex Secretome chip and loaded into

CodePlex Secretome Panel Menu

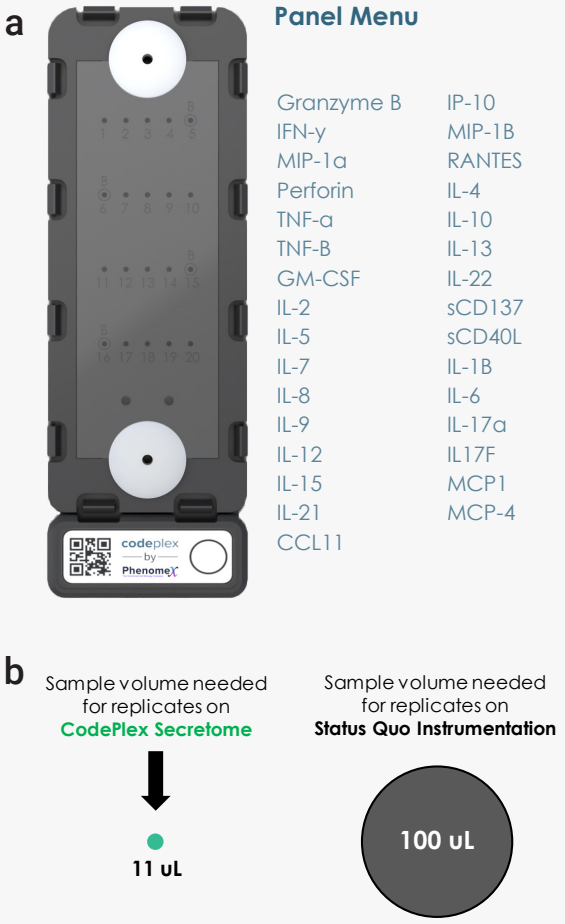

Figure 2 | a) The CodePlex Secretome Adaptive Immune Panel targets 30+ cytokines in bulk, automated on the IsoLight system, and can selectively run eight conditions a chip in "MacroChambers" across eight chips on a single run. b) Easily run replicates with a small sample volume: 5.5 uL per microwell (11 uL per sample replicate)

Conventional Multiplexed Bulk Cytokine Platform vs the IsoLight Automated Benchtop System

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

ISOLIGHT 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.

ISOLIGHT STEPS FOR FUNCTIONAL IMMUNE PROFILING

Figure 3 | The IsoLight automated benchtop system 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, and data is fully analyzed via IsoSpeak software.

the IsoLight system. None of the ELISA procedures are performed by the user, as they are completely automated within the IsoLight. With the IsoSpeak software suite, data is instantly fully analyzed and visualized, saving both time and costs.

Understanding tumor metastasis with CodePlex Secretome

Cancer cells in the tumor microenvironment secrete cytokines that interact with other cells, such as immune cells, in a 3D extracellular matrix, which facilitates intercellular communications and jointly moderate pathophysiological processes, "including cancer-induced angiogenesis and metastasis."[1] Secretomic profiles of cancer cells evolve as they multiply and become more invasive, which suggests that secreted factors may be associated with proliferation and migration.

Published in *Nature Communications*, researchers utilized Bruker's technology to gain insight into whether soluble molecules secreted by cells were responsible for controlling the cell-density-dependent migration. Researchers discovered that the soluble molecules secreted by the matrix embedded cancer cells promoted improved cell migration.

Secreted Cytokines Promote Tumor Metastasis

	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

	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

Figure 4 | 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.

Researchers used CodePlex to identify which soluble factor or factors were driving increased mobility by measuring and analyzing the secretomic profiles of the HT1080 and MDA-MB-321 cells in 3D matrices at varying densities (Figure 4). Bruker technology, which simultaneously measured the concentration of 24 soluble molecules. Both cytokines IL-6 and IL-8 were secreted at high concentrations in a specific ratio and density-dependent manner, while proteins typically associated with promoting tumor metastasis and progression were not elevated, suggesting that both of these cytokines are responsible for driving the density-dependent cell migration within 3D matrices. This study reveals a possible mechanism promoting tumor cell migration while inferring an approach to reduce metastatic capability of tumor cells.

The IsoLight: A Hub for Comprehensive Functional Profiling of Each Cell Type Across a Large Assay Menu of Single-Cell and Population Chip and Software Products

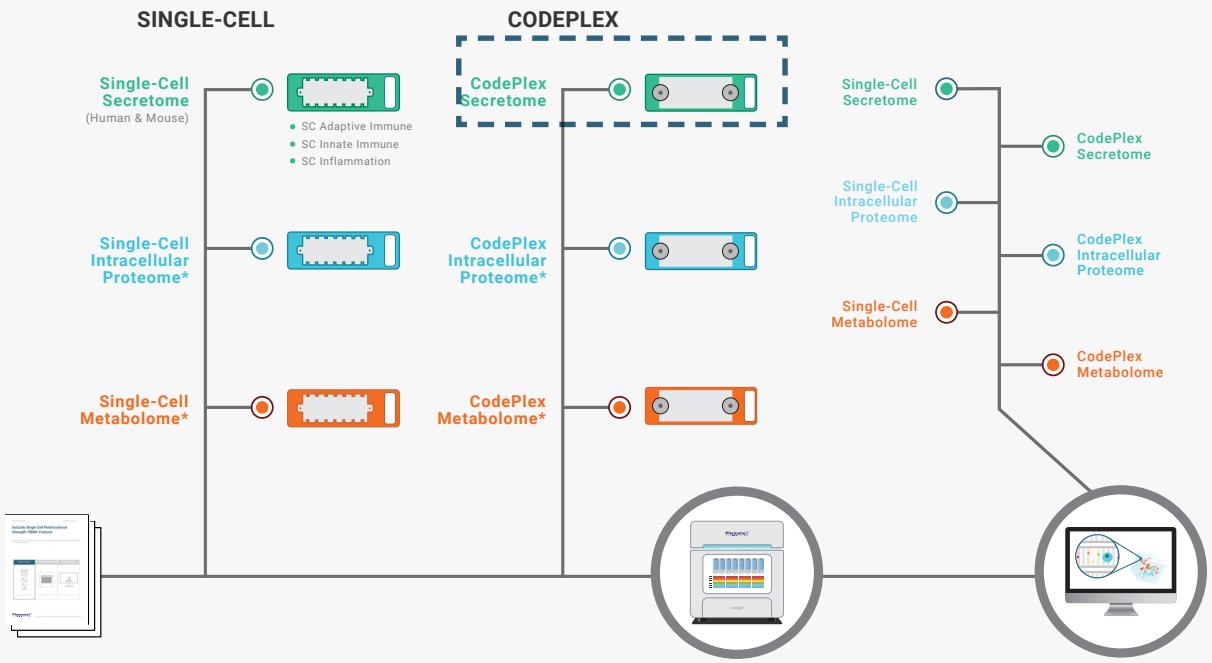

Figure 5 | The IsoLight system serves as a proteomic hub, enabling both single-cell and population cytokine assays for a variety of cell types on a single system

Democratizing cellular proteomics to accelerate immune medicine

The IsoLight is a hub for comprehensive functional profiling of each cell type across a large assay menu of single-cell and population chip and software products (Figure 5). Bruker's automated suite of life sciences products are built to power the future, which lies in immune medicines. Using the automated IsoLight system, Bruker is revealing true immune biology, unlocking development, and accelerating biomarker discovery.

Conclusion

- Bruker's CodePlex Secretome solution provides clear benefits over conventional multiplexed bulk ELISA platforms
- The CodePlex Secretome solution provides automated highly multiplexed proteomics with only five minutes of hands-on time
- Understanding how secreted cytokines promote tumor metastasis provides a potential strategy towards decreasing the metastatic capacity of cancer cells

References

1. 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