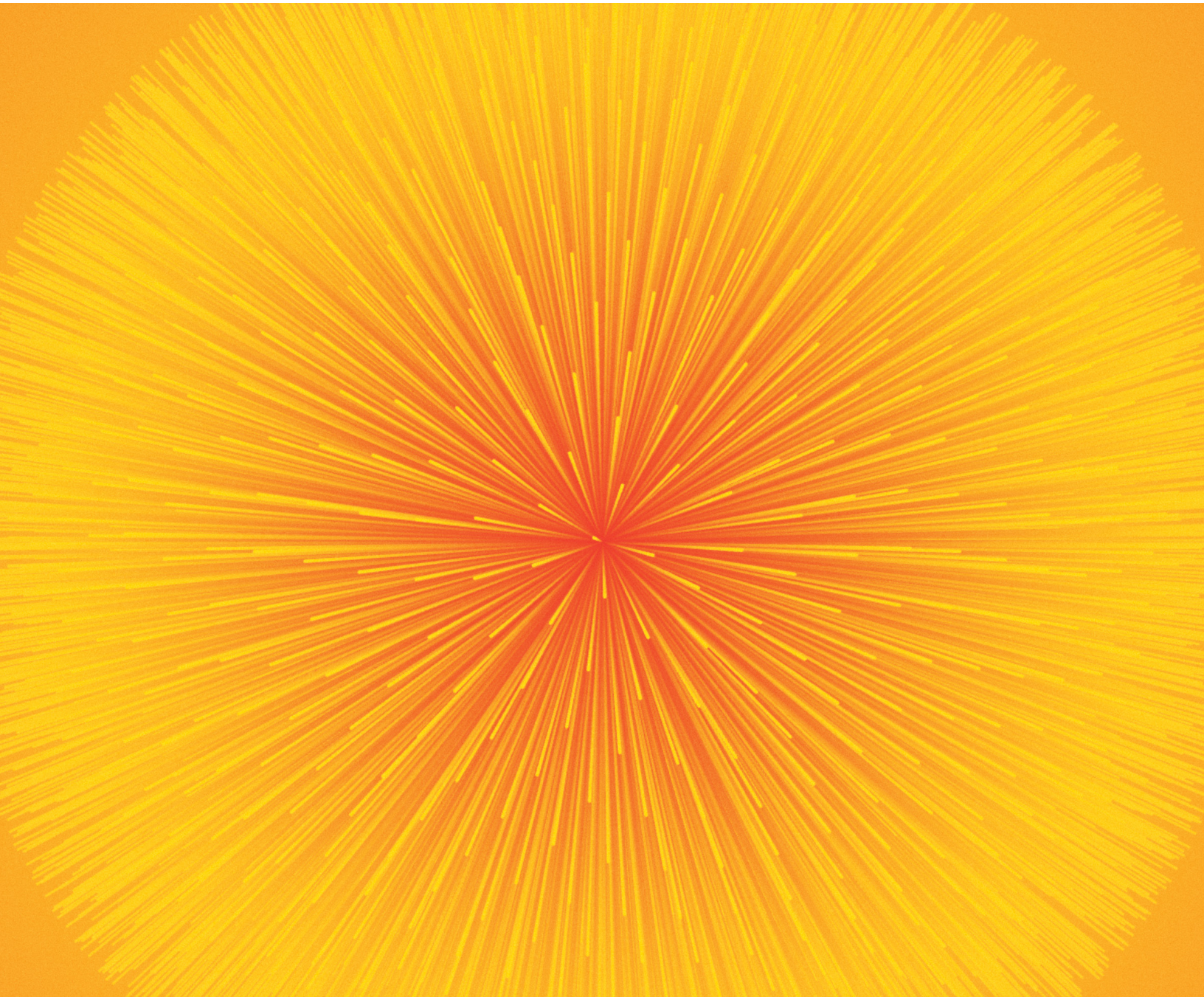
Olink®

Olink® Explore HT:
Discover more biology with confidence



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Introduction

Olink Explore HT is a next-generation proteomics platform designed for high-throughput protein biomarker discovery at any scale with outstanding analytical performance.

Building on the proven success of Olink® Explore 3072, Olink Explore HT expands the previous library by **85%**, to approximately 5,400 human proteins, offering broader proteome coverage while upholding stringent data quality standards.

Olink Explore HT features a streamlined and automated workflow designed to enable faster turnaround times and reduced reagent consumption. Complementing this are NPX™ Map and Olink® Analyze, which are tailored for high-throughput data acquisition and analysis, empowering users to extract actionable biological insights more efficiently.

This white paper aims to provide a comprehensive overview of Olink Explore HT, focusing on the design of its biomarker library and critical analytical parameters, including detectability, specificity, and precision. It also addresses the concordance and bridging between Olink Explore HT and Olink Explore 3072, ensuring seamless integration and comparability. By offering these insights, the white paper equips researchers with essential information to effectively plan and execute their proteomics studies.

Comprehensive library expansion enabling additional biological insights

Carefully curated library with improved coverage

The library of approximately 5,400 protein assays enables deeper and broader proteome analysis than ever before. It was carefully designed to provide more comprehensive and actionable biological insights, drawing from various criteria such as annotations, publications, clinical trials, drug targets, and requests from the research community. As a result, the expanded Olink Explore HT library provides more extensive coverage of key biomarker categories (Figure 1) and biological pathways (Figure 2).

- **Low-abundant** immune system-related proteins linked to a wide range of human diseases.
- **Proteins actively secreted** into the blood, highly relevant for clinical diagnostics and precision medicine.
- **Tissue-elevated** proteins that may be released into the blood due to cell death or tissue damage.
- Approved and candidate **drug targets**.

Figure 1. Extensive and comprehensive coverage across all major biomarker categories.

The library covers an impressive 85% of all 2,931 human biological pathways in the Reactome database (Reactome Database V.91), including 100% of the top-level biological pathways (level 0) and an excellent representation of lower-level pathways, see Figure 2.

This extensive pathway coverage allows researchers to explore biological processes with both breadth and depth.

Broadened library scope that recapitulates the human proteome

The expansion of the Olink Explore HT library shifts the focus from increasing the depth of the existing secretory protein library to broadening its scope by including a greater diversity of nuclear, intracellular, and tissue-enriched proteins. Consequently, this library expansion strategy enables more unbiased exploration of diverse protein types, such as those beyond the commonly studied blood proteins, providing opportunities to uncover unexpected disease associations.

As illustrated in Figure 3, the new library has been refined to more accurately recapitulate the distribution of protein subcellular locations covered by the human proteome. Importantly, these proteins can offer great value in exploring novel biology reflecting tissue damage during a disease process, in addition to other sample matrices, such as cell and tissue lysates, where intracellular proteins are more commonly found.

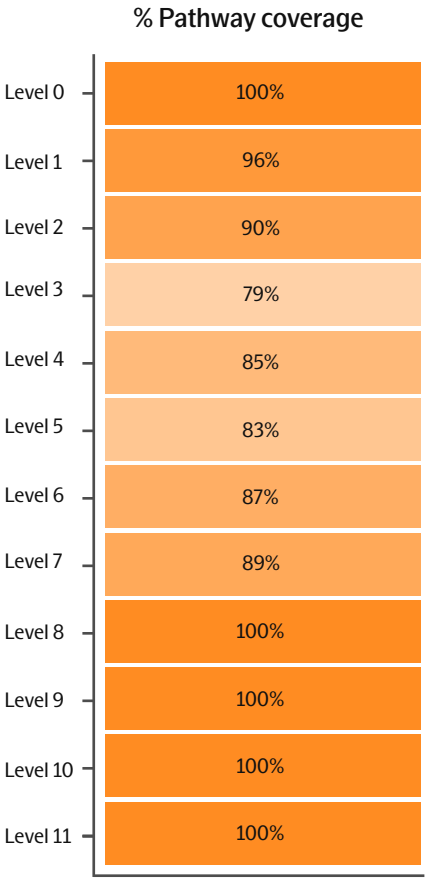


Figure 2. Heatmap illustrating the Reactome pathway coverage of the Olink Explore HT library across all 12 pathway levels.

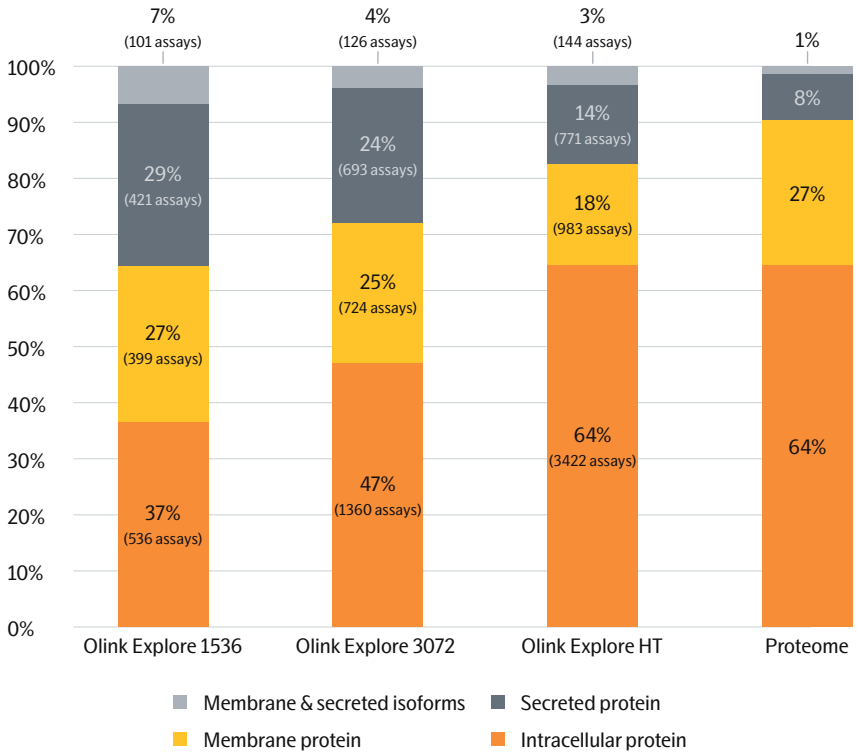


Figure 3. The evolution of the library content from Olink Explore 1536 to Olink Explore HT highlights a significant expansion in library size, providing more comprehensive coverage of the diverse protein types within the human proteome.

The number indicates the actual assay number, note that not all assays in the different products have a subcellular location annotated in HPA.

Discover more biology with confidence: Olink Explore HT case studies

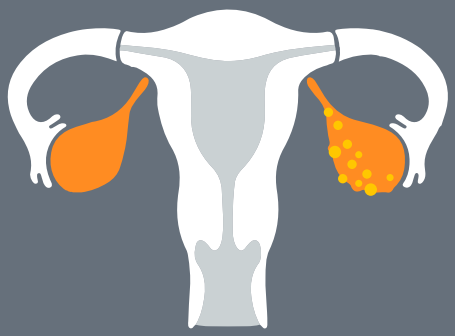
Case study 1 – Ovarian cancer:

Discovery of new ovarian cancer tumor differentiation biomarkers

Principle Investigator: **Ulf Gyllensten, Ph.D.,** Senior Professor, Department of Immunology, Genetics and Pathology, Uppsala University.

Ovarian cancer has the highest mortality among gynecological cancers, with surgery often used for final diagnosis. Accurate molecular tests are needed when imaging techniques are inconclusive.

In a recent biomarker discovery study using Olink Explore HT, the intracellular protein L1RE1, newly introduced in the Olink Explore HT library and absent in the Olink Explore 3072 library, was identified as a novel and significant marker for distinguishing between benign conditions and late-stage ovarian cancer. L1RE1 was undetectable in healthy control groups but detected in the plasma of ovarian cancer patients. This finding underscores its potential for clinical applications as a component of a new biomarker signature, offering a means to significantly reduce the need for diagnostic surgery in suspected ovarian cancer cases.



CASE STUDY

"Advancing biomarker discovery in ovarian cancer with Olink® Explore HT"

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WEBINAR

"Protein Biomarkers for Tumor Differentiation: Insights from Olink® Explore HT"

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Discover more biology with confidence: Olink Explore HT case studies

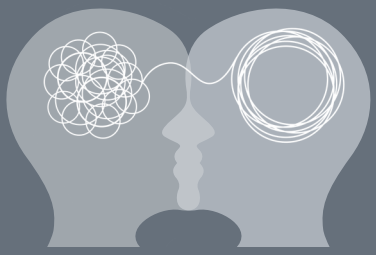
Case study 2 – Postpartum depression:

Discovery of new postpartum depression biomarkers

Principle Investigator: **Clarissa Yates, Ph.D.,** CEO & Founder, Ketim Therapeutics.

Postpartum depression (PPD) is a serious mental health condition affecting mothers after childbirth, with significant consequences for maternal well-being, infant development, and family dynamics. Currently, there is a lack of knowledge of the fundamental biological mechanisms underlying PPD, hindering effective diagnosis and treatment.

In a recent biomarker discovery pilot study investigating a cohort of 50 mothers diagnosed with PPD using Olink Explore HT, three novel protein biomarkers were identified with significant disease associations. These biomarkers are newly introduced in the Olink Explore HT library and absent in the Olink Explore 3072 library. They represent a unique discovery that sheds light on previously unknown disease mechanisms underlying PPD. The identified biomarkers are currently undergoing validation and hold the potential to serve as distinctive tools for early detection and treatment response monitoring of PPD.



WEBINAR

"Charting the Future of Mental Health Precision Medicine with Proteomics"

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Robust and biologically relevant detectability

Evaluation of Olink Explore HT detectability

The detectability of an assay refers to its ability to identify and measure its target analyte above background noise. This depends on the assay's sensitivity (the ability to detect low concentrations of the analyte), dynamic range (the ability to measure analyte concentrations across a wide range), and specificity (the ability to exclusively measure the intended analyte without cross-reacting with other analytes).

During the development and validation of Olink Explore HT, calibration curves were established for approximately 5,400 assays using serial dilutions of recombinant proteins (Figure 4). These curves demonstrated that each assay could detect its intended target protein while providing information on assay sensitivity and dynamic range. As a result, Explore HT offers a broad dynamic range spanning over 10 orders of magnitude(fg/mL to mg/mL), enabling the accurate detection of both high- and very low-abundance proteins.

The detectability of Olink Explore HT assays was also evaluated using biological samples from two independent studies, including 516 plasma samples from ovarian cancer patients and 164 cell lysates. The results showed that 97% of the assays successfully detected their target proteins above background levels (above the limit of detection) in at least five samples, confirming that these assays are functional and capable of detecting their target proteins in biological samples (Figure 5).

VALIDATION DATA

"Olink Explore HT validation methods and results"

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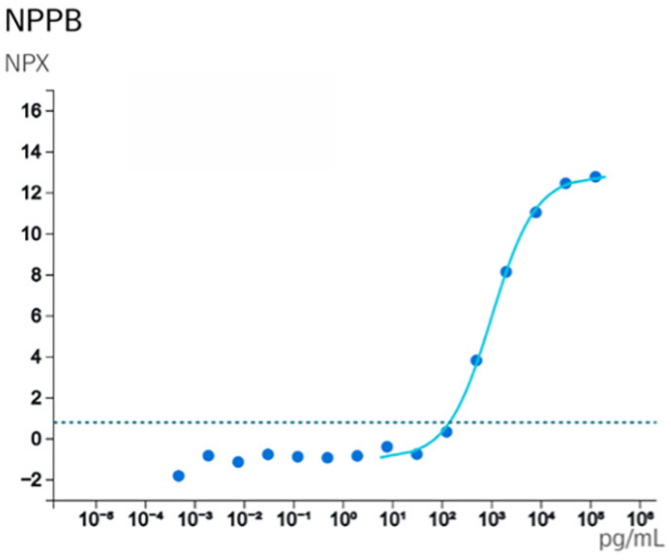


Figure 4. An example of the protein calibration curve for assay detecting NPPB (UniProt: P16860).

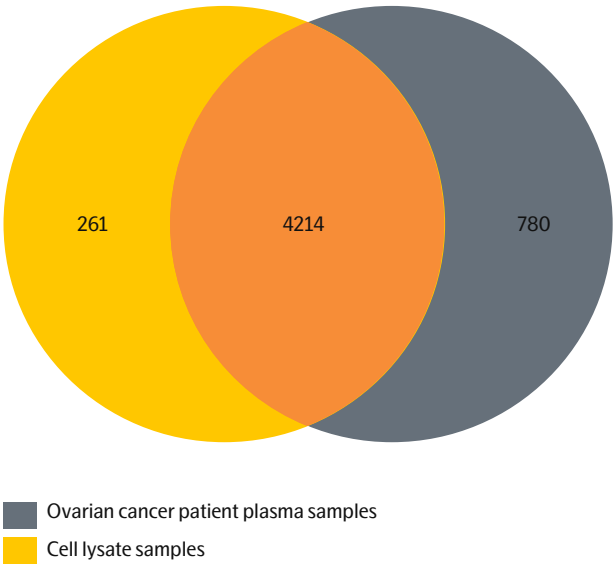


Figure 5. The detectability of Olink Explore HT was assessed in two studies using cell lysates (N=164) and ovarian cancer patient plasma samples (N=516). Overall, 97% of assays detected target proteins in at least 5 samples. The detectability increases to 99.7% with a 1-sample threshold (data not shown)

Robust and biologically relevant detectability

The detectability of Olink Explore HT captures biology

Detectability in a biological context refers to the frequency with which a protein is detected above LOD (Limit of Detection) in biological samples based on a specified threshold. For example, if the threshold is set at >50% of the samples and the detectability of a sample set is calculated to be 55%, this means that 55% of the assays are detected above the LOD in more than 50% of the samples. Thresholds other than 50% can be selected based on the purpose of the investigation, and the chosen threshold will influence the calculated detectability.

Detectability in a biological context depends on both the assay's technical performance and the natural abundance of the protein in biological samples, as well as any biological variation across samples. Consequently, it often varies depending on the sample type, the cohort, and individual biological differences, as shown in Figure 6.

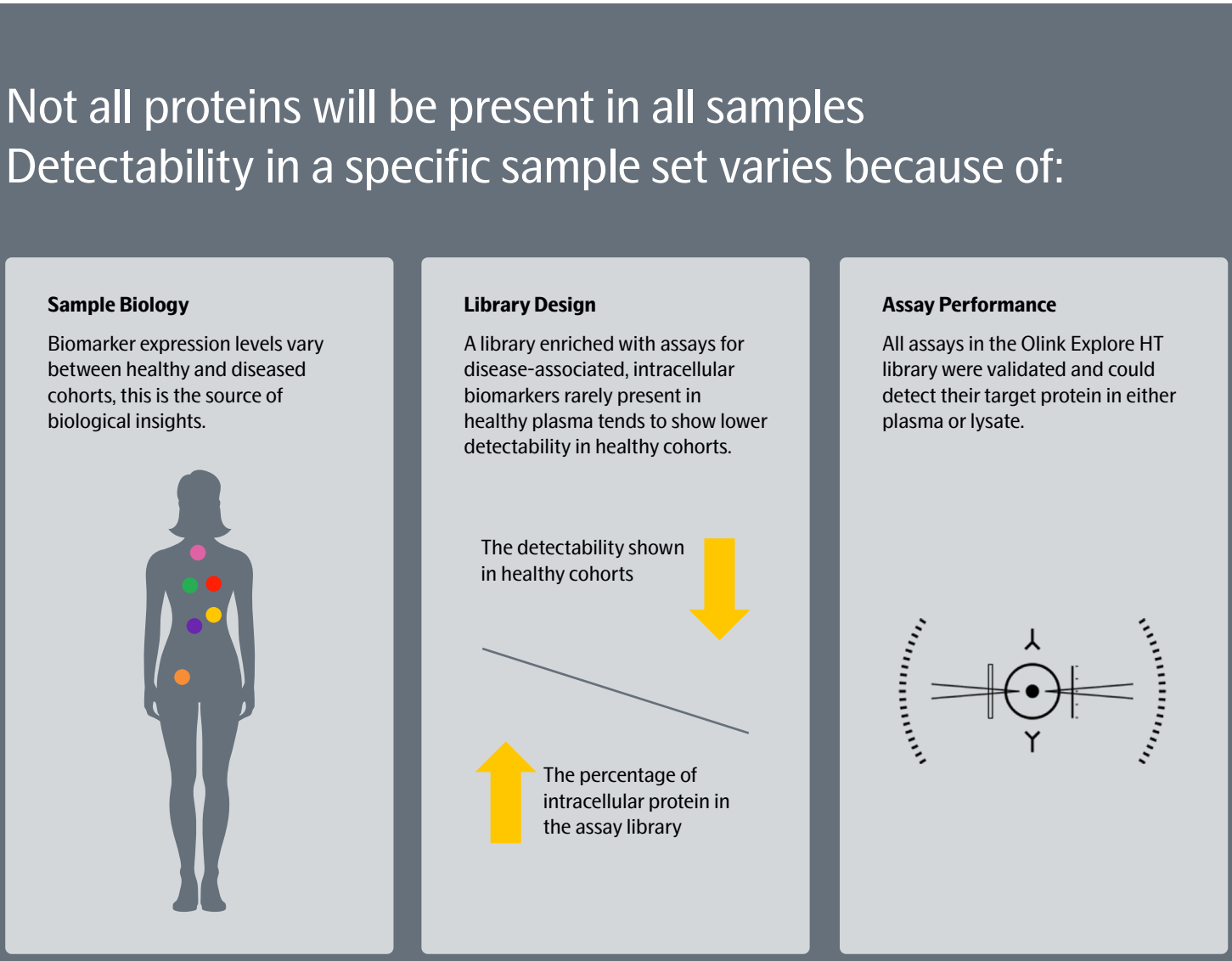


Figure 6. Factors influencing detectability in biological samples.

Robust and biologically relevant detectability

The detectability of Olink Explore HT captures biology

The Olink Explore HT library contains a high proportion of intracellular and tissue-specific proteins that may not be present in healthy blood samples but enter circulation due to cell death or tissue damage. Consequently, not all proteins will be present in every sample, leading to variability in detectability across sample sets. This variability is a valuable source of new biological insights, as it can uncover patterns linked to specific biological conditions or disease states. Figure 7 illustrates how the detectability in biological samples could vary using data from two independent studies.

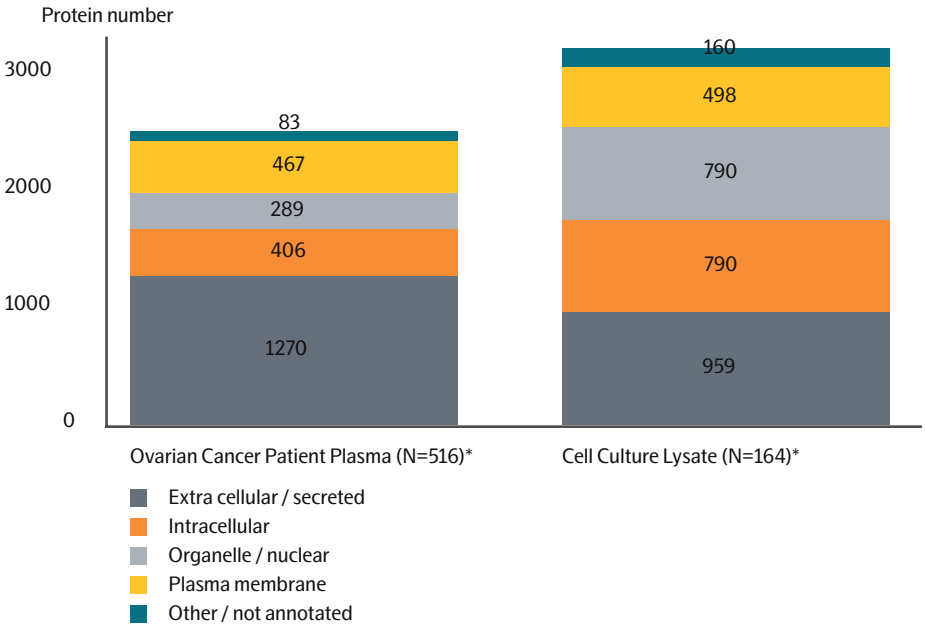


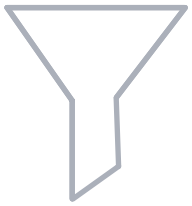
Figure 7. Assessment of Olink Explore HT's detectability for various protein categories, in two independent studies using cell lysates and ovarian cancer patient plasma samples. This illustrates how biological detectability is significantly influenced by both the protein type and sample matrix type. *Threshold: minimum 30% samples

In summary, the detectability of a proteomics platform is influenced by both its technical performance and the biological characteristics of the samples analyzed, particularly regarding the types of proteins being targeted and sample matrices (Figure 7). Olink Explore HT has demonstrated biologically relevant and reliable detectability, accurately reflecting the biology of the analyzed samples.

Olink's Recommendations for LOD Calculation and Filtering

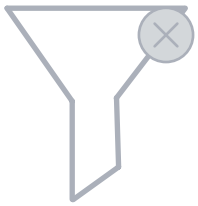
The calculation of the limit of detection (LOD) directly impacts detectability and precision calculation. Therefore, we recommend reviewing the following LOD filtering and calculation guidelines to ensure the data analysis strategy aligns with the research objectives.

LOD filtering for technical evaluations:



- Reproducibility measurements
- Concordance assessments
- Detectability calculations

Keep values below LOD to extract more biology:



- Exploratory investigations and statistical analysis
- Capture more biology
- Avoid bias

LOD CALCULATIONS

Further details on LOD calculations are described in the LOD tutorial on the Olink® Analyze CRAN page

LEARN MORE

Please note:

Olink recommends including all data in downstream statistical analysis (no LOD filtering) to allow for novel discoveries representative of pathological conditions. LOD can, however, be informative for technical metrics, Olink recommends that CV calculations utilize data > LOD. For customers interested in LOD, the project-specific LOD can be calculated using the Olink Analyze olink_lod() function in R.

Uncompromised analytical performance

The unique features of PEA technology ensure consistent and high analytical performance, regardless of the multiplex level, providing researchers with a reliable tool to explore biology with confidence. Olink Explore HT delivers the same high-quality data as previous generations of Explore or other PEA platforms, despite the significant increase in library size.

Outstanding specificity through DNA-coupled, dual-antibody recognition

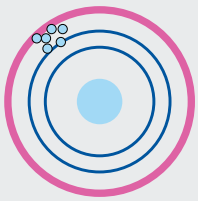
Besides a well-curated biomarker library, discovering more biology also requires accuracy, which means measuring the correct protein target (high specificity) precisely (high precision).

Reflect technical performance and true biology

Precise and specific

- Measure the right protein
- Minimize false positives

Reflects technical performance only



Precise but not specific

- Consistently off-target
- Incorrect conclusions

Figure 8. High specificity is the most critical analytical parameter in biomarker assays.

Consequences of poor specificity:

Misidentification and false biological signatures:

Incorrect protein identification can lead to erroneous conclusions, diverting research efforts and resulting in costly failures.

Delayed discoveries:

Lack of specificity can impede progress in understanding disease mechanisms, slowing the development of effective treatments and delaying improved patient outcomes.

Wasted resources and misallocation of future resources:

Time, funding, and precious biological samples may be expended on studies that yield incorrect or misleading data. Future efforts may become focused on irrelevant proteins or biological pathways, hindering advancement and wasting valuable resources.

Specificity stands as the most critical analytical parameter in biomarker assays. Without high specificity, even precise measurements can lead to inaccurate quantification and misidentification of the target proteins and result in substantial negative consequences.

The high specificity of the Olink platforms is rooted in its unique DNA-coupled, dual-antibody recognition, a core feature of the Proximity Extension Assay (PEA). This approach, based on the correct hybridization of complementary oligonucleotides coupled to the correct pair of matched antibodies, ensures that only the target proteins are detected, significantly reducing cross-reactivity and false positives. During product development, specificity is rigorously validated through extensive specificity screening and un-specific binding challenges, confirming that the assay can reliably differentiate target proteins from structurally similar molecules.

This robust specificity has made the Olink platform the preferred choice for global proteogenomics initiatives, especially in identifying protein quantitative trait loci (pQTLs).

WHITE PAPER

"PEA: Exceptional specificity in a high multiplex format"

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Consistently high precision at any scale

The precision of PEA has been preserved as the number of measured proteins increased across Olink platforms, from 21 to approximately 5400, as shown in Figure 9. Olink Explore HT demonstrated a median intra-CV of 8%, similar to Olink Explore 3072. This high precision ensures accurate and consistent quantification of a wide range of proteins, minimizing variability and providing reliable data for robust insights for diverse research applications.

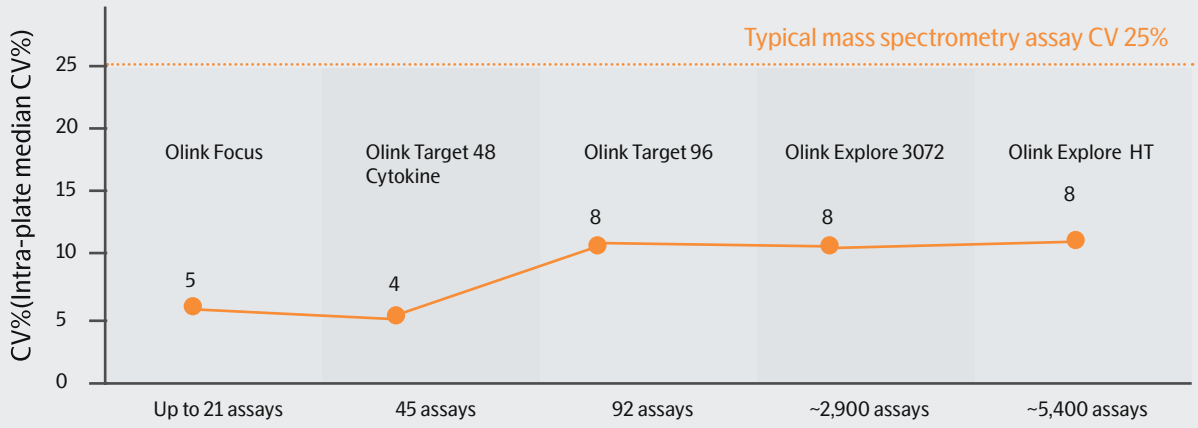

Figure 9. Precision comparison between different Olink platforms (for data >LOD).

The precision of Olink Explore 3072 and Olink Explore HT was further compared in two independent studies: the Olink® Concordance Test and the ovarian cancer case study.

The Olink® Concordance Test was conducted by Olink Analysis Service Boston. As shown in Figure 10, Olink Explore HT has a median intra-CV of 8.4% compared to 9.4% for Olink Explore 3072, and a median inter-CV of 7.2% versus 11.7%.

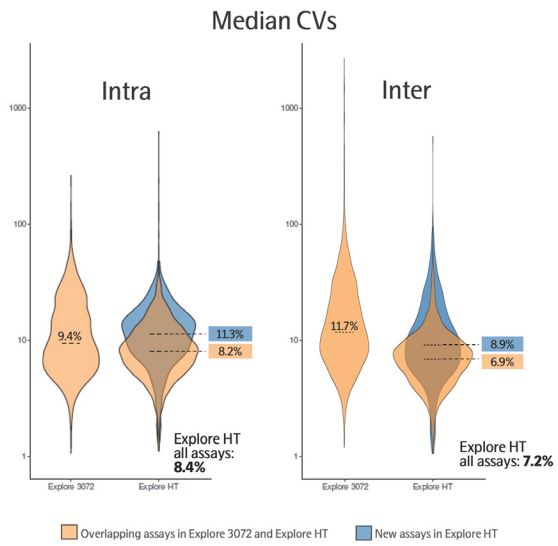

Figure 10. Precision comparison with the Olink Concordance Test using 8 pools of human plasma samples. (Data>LOD).

Precision data from the ovarian cancer case study, conducted in collaboration with Uppsala University, supports similar results. Inter-CVs from 42 Sample Controls (across 14 plates) on Olink Explore HT were compared to those from 182 Sample Controls (across 91 plates) on Olink Explore 3072, see details in Figure 11.

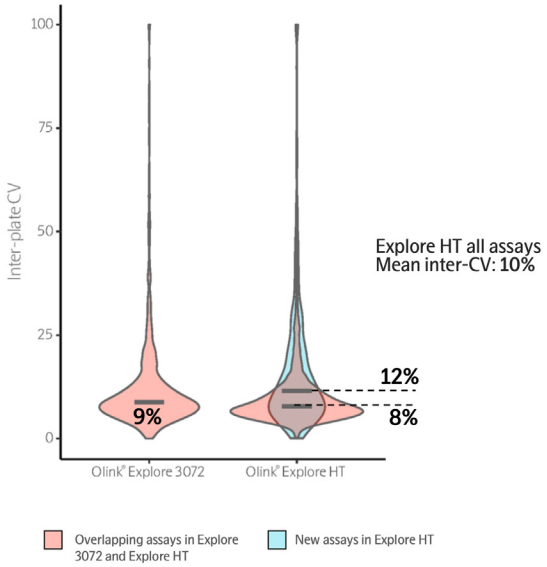

Figure 11. Precision comparison between Olink Explore 3072 and Olink Explore HT in the ovarian cancer study. (Data>LOD).

These results demonstrated that Olink Explore HT offers precision that is as good as or better than that of Olink Explore 3072. These findings confirm that Olink Explore HT sets industry-leading standards for precision, reinforcing its reliability and robustness for high-performance proteomics research.

Seamless Data Integration and Comparison Between Olink Explore 3072 and Olink Explore HT

High concordance between the Olink Explore 3072 and Olink Explore HT platforms, combined with a robust bridge normalization strategy, is crucial for enabling users to confidently and seamlessly integrate data generated from both platforms across different experiments, ensuring consistency and comparability across studies.

Concordance study

Around 2,800 overlapping assays were analyzed for correlation using 50 EDTA plasma samples (including healthy and disease samples) processed in three separate runs at both Olink Analysis Service locations in Boston and Uppsala. The analysis yielded a mean correlation of 0.9 and a median correlation of 0.97, as shown in Figure 12. These results highlight the high consistency between the Olink Explore 3072 and HT products and demonstrate robust performance across different testing sites.

Concordance was also evaluated using patient samples in an ovarian cancer study. As shown in Figure 13, the correlation of effect size between in the two products was approximately 0.9. These results further validate the high concordance level of Olink Explore HT assays in practical applications.

These results illustrate the high concordance between the Olink Explore 3072 and Olink Explore HT platforms which provides a strong foundation for seamless data integration and comparison.

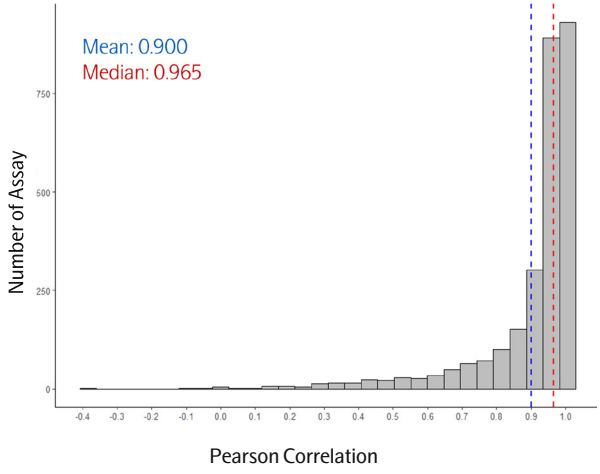

Figure 12. Concordance between Olink Explore 3072 and Olink Explore HT across the ~2,800 overlapping assays.

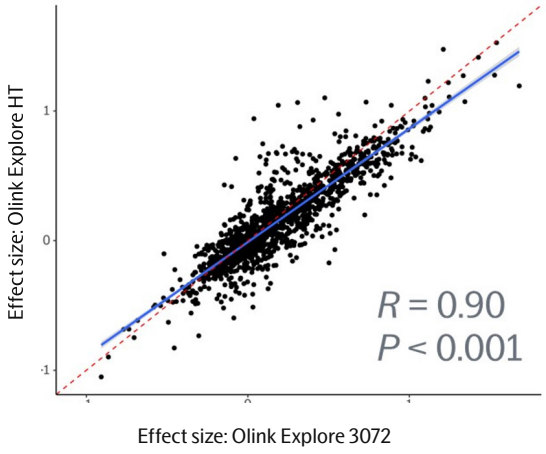

Figure 13. The correlation analysis of effect size on effect size (DNPX) between benign and ovarian cancer groups.

Seamless Data Integration and Comparison Between Olink Explore 3072 and Olink Explore HT

Data bridge normalization

When generating data from multiple runs carried out on separate occasions, it is important to acknowledge the risk of potential technical variation that may risk confounding measurements separated in time, commonly known as the batch effects. By adhering to strict laboratory Standard Operating Procedures (SOPs), technical variance can be reduced.

However, as with any relative quantification method, the potential for batch effects should not be overlooked, regardless of the assay mechanism, to ensure a confident comparison of results across different experiments. Batch effects can be further minimized through bridge normalization, which leverages overlapping reference samples, commonly referred to as bridging samples.

Similarly, when generating data on two different platforms such as Olink Explore 3072 and Olink Explore HT, bridging samples can be used for the normalization of data across platforms. Recognizing this importance, the Olink Analyze R package provides a function that allows users to perform bridge normalization of a previous Olink Explore 3072 study to Olink Explore HT study or to simply perform bridge normalization of data generated within the same platform on different occasions through the use of overlapping reference samples.

TUTORIAL

"Olink Explore 3072 to Olink Explore HT bridging tutorial"

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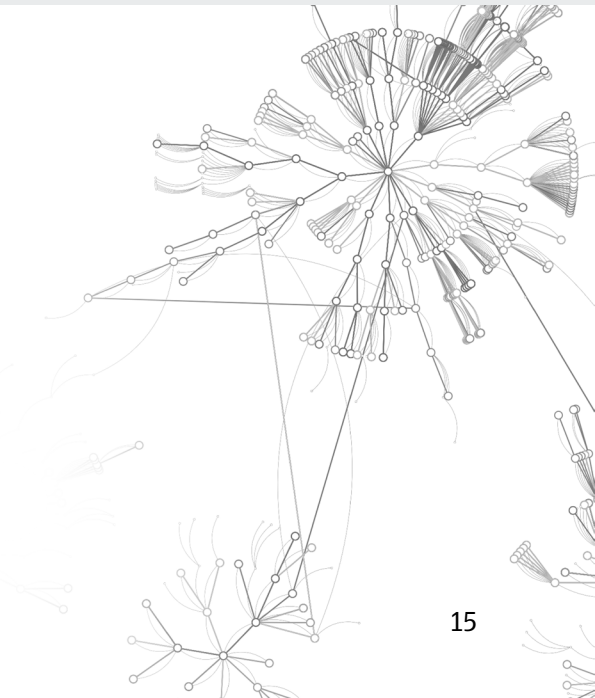
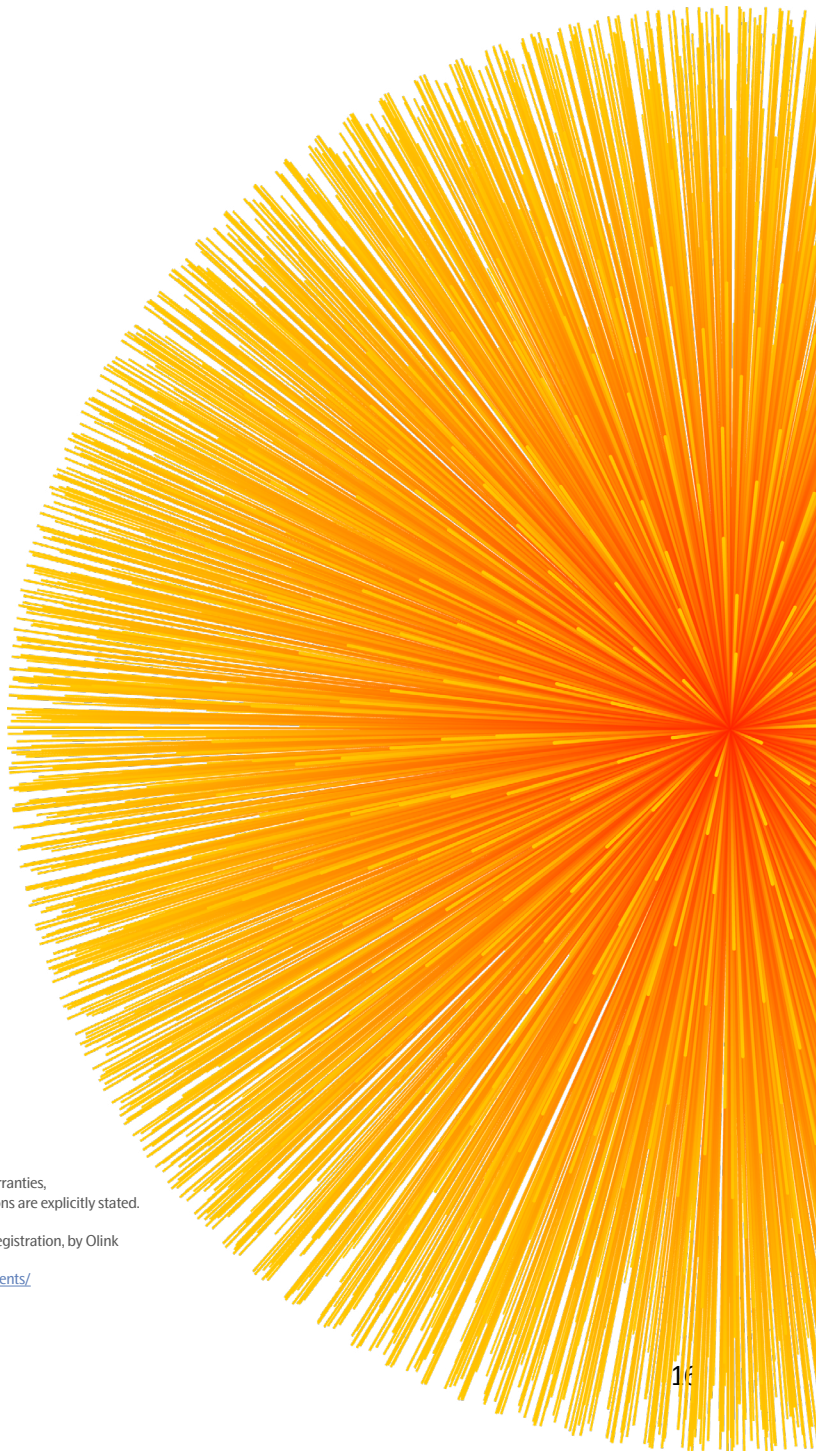
Conclusion

Olink Explore HT represents a groundbreaking advancement in proteomics, offering an unparalleled combination of a meticulously designed protein library, exceptional specificity and precision, unmatched scalability, and high-throughput capacity.

It empowers scientists to discover biology with confidence, uncovering critical insights into complex diseases, accelerating biomarker discovery, and advancing precision medicine research at an unprecedented scale. It sets a new standard for high-resolution protein analysis, driving transformative progress in life science research.





www.olink.com

Olink Proteomics, Salagatan 16F , SE-753 30 Uppsala, Sweden

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