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Proteomics

Olink™ Reveal grant application support file

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Contents

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How to use this document

This document is designed as a modular resource to support the development of grant applications involving Olink Reveal proteomics. It provides language that can be adapted and incorporated into different sections of a grant proposal.

The content is intended to help investigators:

- Clearly articulate the scientific rationale for proteomics
- Describe the technology and its advantages
- Demonstrate rigor, reproducibility, and feasibility
- Support study design, implementation, and data analysis plans

Rather than being used in full, sections should be selected and tailored based on the specific grant requirements and study design.

In-house vs. Analysis Services workflows

This document includes content for both in-house implementation and service-based workflows.

- For Analysis Services (AS) studies, omit:
  - Section 8 (Workflow)
  - Section 9 (NGS readout)
  - Section 10 (Data generation)
  - Section 12 (Training & implementation)

Instead, use:

- Section 13 (Analysis Services)
- Or the included reference to Certified Service Providers (CSPs)

Additional support

For study-specific guidance, investigators are encouraged to consult Olink sales representatives to ensure alignment with project requirements.

1. Justification / value of proteomics

Proteomics provides a critical layer of biological insight beyond genomics and transcriptomics by directly measuring proteins—the functional effectors of biological systems. While DNA represents a largely static blueprint of inherited risk, protein expression reflects the dynamic, real-time state of biological processes, capturing disease onset, progression, and response to therapy.

As the primary mediators of cellular function and direct targets of most therapeutics, proteins offer a more immediate and actionable link to phenotype than upstream molecular layers. High-throughput proteomics enables large-scale interrogation of complex biological systems, supporting discovery of disease mechanisms, biomarkers, and therapeutic targets.

Importantly, proteomics addresses key challenges in precision medicine by enabling identification of the right drug (through direct target measurement), the right patient (via protein-based stratification), and the right time for intervention (through dynamic biomarker changes).

Large-scale population proteomics studies further demonstrate that integrating proteomic and genomic data enhances causal inference, improves disease prediction, and accelerates translation. This proteogenomic approach enables identification of biologically relevant pathways and supports development of predictive models that better reflect real-world disease biology.

2. Value of Olink platform

The broader application of proteomics has historically been limited by challenges in analytical specificity, sensitivity across a wide dynamic range, scalability, and reproducibility. Traditional approaches often require trade-offs between depth, throughput, and robustness, and may rely on complex workflows, large sample volumes, or limited multiplexing capacity. In addition, cross-reactivity and nonspecific binding can compromise data quality, while variability across platforms can hinder reproducibility and complicate the transition from discovery to clinical translation.

Olink's Proximity Extension Assay (PEA™) addresses these challenges, as described in the next section. By enabling consistent, high-quality data generation across the full research continuum—from discovery to clinical translation—using the same core technology, the need to switch between platforms is eliminated, reducing variability and improving efficiency.

The platform is optimized for minimal sample input while maintaining high sensitivity and specificity, enabling the detection of low-abundance proteins across a broad dynamic range and supporting studies with limited sample availability. Standardized workflows and integrated data analysis further ensure robust, reproducible results across laboratories, timepoints, and large-scale cohorts.

Olink technology has been applied in nearly 4,000 publications and large-scale initiatives such as UK Biobank Pharma Proteomics Project, as well as in pharmaceutical clinical trials, demonstrating its value in enabling reproducible, high-impact biological insights at scale. Studies using Olink platforms have shown the ability to:

- Identify disease drivers and underlying biological mechanisms[1-3]
- Improve patient stratification and reduce clinical trial sizes[4-6]
- Increase predictive power across multiple disease areas[7-9]
- Accelerate the transition from biomarker discovery to clinical validation[10-14]

3. Olink Technology Overview

Olink's platform is based on the proprietary Proximity Extension Assay (PEA™) technology, which combines antibody-based protein detection with DNA-based quantification.

In PEA, each target protein is recognized by a pair of antibodies, each conjugated to a unique oligonucleotide. Only when both antibodies specifically bind to the same protein molecule, their attached oligonucleotides hybridize and are extended by DNA polymerase to form a unique DNA barcode (Fig. 1). This barcode is subsequently amplified and quantified using qPCR or next-generation sequencing (NGS).

This 3-layer recognition mechanism provides several key advantages:

- **High specificity:** Signal generation requires simultaneous binding of two antibodies, minimizing cross-reactivity and false positives
- **High sensitivity:** Enables detection of low-abundance proteins across a wide dynamic range
- **Scalability:** Supports multiplexing from tens to thousands of proteins in a single assay with uncompromised performance
- **Minimal sample input:** Requires only microliter volumes of biological samples

The platform delivers robust performance across diverse sample types and study designs, with standardized workflows that ensure reproducibility across sites and timepoints.

In addition, Olink's solutions integrate advanced bioinformatic tools and data analysis platforms, enabling researchers to move efficiently from raw data to biological interpretation. These tools facilitate study design, statistical analysis, and integration with large-scale reference datasets, supporting deeper insights into disease biology and accelerating translational outcomes.

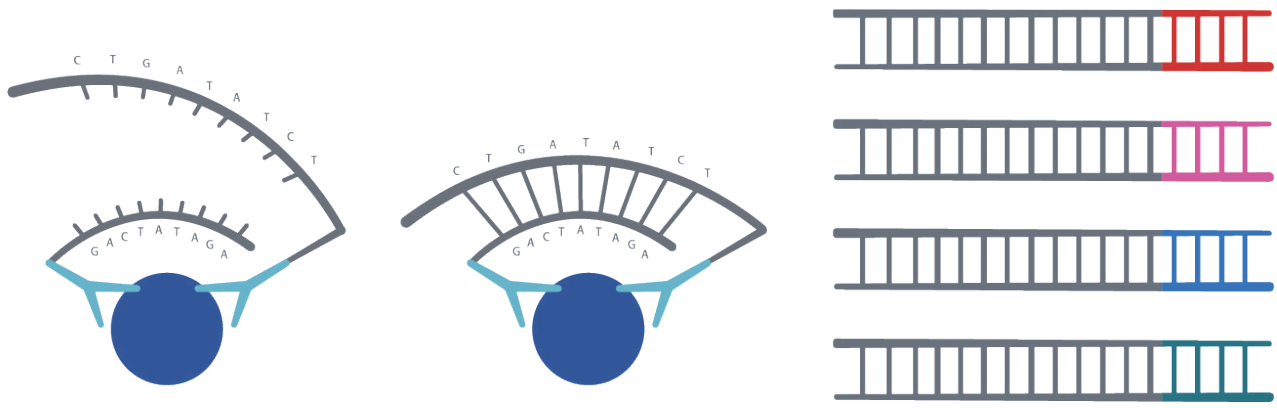

Figure 1: Technology principle of Olink's PEA and DNA barcode generation.

4. Olink Reveal Platform

Olink Reveal is a next-generation proteomics platform enabling accessible protein biomarker discovery, with the simultaneous measurement of ~1000 proteins from minimal sample input (~4 µL of plasma, serum, or other biofluids, but 10 µL are needed for sample preparation). Each kit supports the analysis of up to 86 samples. Olink Reveal is optimized for accessibility and streamlined implementation, utilizing pre-mixed, dried-down reagents and standard laboratory techniques without the need for specialized instrumentation.

The platform is based on Olink's PEA technology combined with next-generation sequencing (NGS) readout.

Analytical performance and rigor

Olink Reveal has undergone a comprehensive multi-step analytical verification process, including a stringent 3-step, 15-factor validation workflow to ensure assay performance and data reliability across multiple biological matrices.

Each assay is evaluated for sensitivity, specificity, and linearity, with defined acceptance criteria applied throughout development.

- **Specificity:** All assays included in Olink Reveal were previously validated to show no measurable cross-reactivity, ensuring accurate protein identification in multiplexed measurements
- **Precision:** Median intra- and inter-plate coefficients of variation are approximately 7.2% and 4.2%, respectively, supporting high reproducibility across runs
- **Sensitivity and dynamic range:** The platform enables detection of proteins at very low abundance levels (down to fg/mL), while maintaining broad biological detectability across diverse protein classes. Assay-specific quantification ranges (LLOQ–ULOQ) are available in supplementary materials.

Reproducibility and cross-platform consistency

Olink Reveal demonstrates strong concordance with other Olink platforms, enabling integration across discovery and validation workflows.

Comparative analyses show high agreement with:

- Olink Explore HT ($R \approx 0.95$)
- Olink Explore 3072 ($R \approx 0.94$)
- Olink Target panels ($R \approx 0.90$)

5. Quality Control

A built-in quality control system incorporates both internal controls (monitoring immunoassay performance, extension, and amplification) and external controls (plate, sample, and negative controls), allowing continuous monitoring of assay performance and data quality at both sample and run levels.

At the core of this system is a combination of internal and external controls, embedded directly within each run. Three internal controls are added to every individual sample to monitor critical steps of the assay: (i) an incubation (immuno) control, which assesses antibody binding and overall assay performance; (ii) an extension control, which evaluates the efficiency of oligonucleotide hybridization and extension; and (iii) an amplification control, which monitors PCR amplification and sequencing readiness (Fig. 2). Together, these controls provide continuous, sample-level verification of assay integrity across all major stages of the PEA workflow.

In addition, each assay plate includes multiple external controls, enabling robust normalization and cross-run comparability. These include five plate controls (used to normalize protein expression values and correct for inter-run variation), three sample controls (used to assess intra- and inter-assay precision), and two negative controls (used to detect background signal and potential contamination). This control structure ensures that both systematic and random sources of technical variation are identified and corrected, supporting reliable comparisons across large datasets and longitudinal studies.

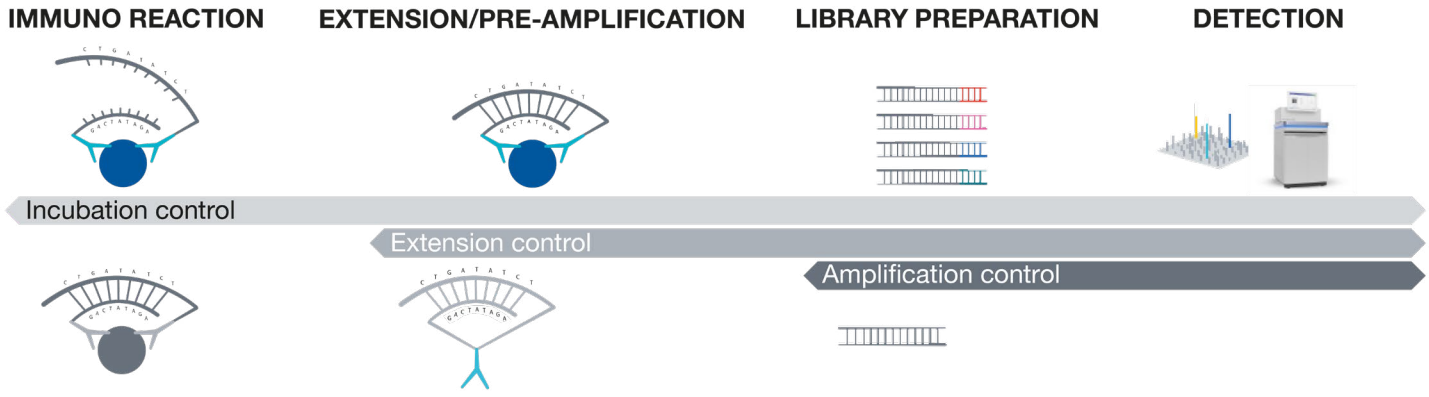

Figure 2: Overview of the Olink Reveal assay workflow and quality control system.

6. Sample types, matrix considerations, and pre-analytical quality control

Olink Reveal has been validated for human plasma and serum, which represent the primary sample matrices for which assay performance characteristics—including sensitivity, specificity, and reproducibility—are established.

While the platform is optimized for these matrices, the underlying PEA–NGS technology is compatible with a range of biological sample types. Assay verification includes evaluation across commonly used matrices such as plasma, serum, cerebrospinal fluid (CSF), tissue, and cell lysates, supporting broader applicability in exploratory research settings.

Alternative matrices: opportunities and considerations

Olink's PEA technology supports a wide range of alternative matrices that enable investigation of biological processes in tissue- or disease-specific contexts, often providing greater mechanistic insight than systemic circulation alone.

However, because Olink assays are optimized for the dynamic range of plasma and serum, matrix-specific considerations are essential. Protein abundance, composition, and interference profiles differ substantially between matrices, and therefore require careful study design and optimization.

Feasibility testing for non-standard sample types

For alternative matrices, a pilot or feasibility study can be performed to evaluate:

- Protein detectability within the matrix
- Compatibility with assay dynamic range
- Dilution linearity and potential hook effects
- Reproducibility and technical variability

These studies ensure that observed signals reflect biological variation rather than matrix-specific artifacts and are particularly important when working with complex matrices such as lysates or low-volume clinical samples.

Non-human species and translational applications

Olink Reveal can potentially be applied to non-human species with some caveats. While assays are primarily developed against human targets, cross-reactivity is often observed due to protein homology, enabling proteomic analysis in preclinical studies.

- Non-human primates show high detectability and comparable data quality
- Mouse models are better supported through dedicated species-specific panels
- Cross-species performance depends on epitope conservation and protein expression, and should be evaluated via feasibility testing

7. Pre-analytical variation and the role of Olink Sample Index (OSI)

A critical factor influencing proteomic data quality across all sample types is pre-analytical variation, which includes variability introduced during sample collection, handling, processing, and storage. Factors such as time to centrifugation, handling temperature, freeze–thaw cycles, and chemical interference can significantly impact measured protein levels.

To address this, the workflow incorporates the Olink Sample Index (OSI), a quantitative, sample-level metric designed to assess and account for pre-analytical variation.

OSI provides:

- A data-driven estimate of sample handling conditions, including time to centrifugation and preparation temperature
- A standardized score (0–1) reflecting the degree of pre-analytical variation per sample
- Both continuous and categorical outputs, enabling integration into statistical models and quality assessment workflows

Importantly, OSI is not a pass/fail metric, but rather a tool to identify and quantify sources of technical variability that may influence biological interpretation. By incorporating OSI into downstream analyses (e.g., as a covariate in regression models), researchers can:

- Reduce noise from sample handling variability
- Improve statistical accuracy
- Increase confidence that identified signals reflect true biology rather than technical artifacts

8. Standardized and accessible workflow (for In-house implementation only)

A critical determinant of data quality in proteomics studies is the ability to ensure consistent sample handling while minimizing technical variability. Olink Reveal addresses this through a standardized, streamlined workflow designed for implementation using standard laboratory equipment and techniques.

Simplified and standardized workflow

The Olink Reveal workflow integrates all key steps from sample preparation to sequencing within a structured two-day protocol (Fig. 3), including:

- Sample dilution and transfer
- Multiplex immunoassay incubation
- PCR amplification and indexing
- Library pooling and purification
- Quality control and NGS readout

This workflow is illustrated in the protocol overview (Day 1–Day 2), where samples are incubated with antibody pairs, followed by DNA barcode generation and sequencing-based detection.

Ease of implementation

Olink Reveal is designed for manual pipetting workflows, without the need for specialized automation systems, while remaining compatible with optional liquid handling platforms for increased throughput.

Key features supporting reproducibility include:

- Pre-mixed, dried-down reagents to reduce preparation variability
- Standardized plate-based workflow
- Controlled laboratory environment

To maintain assay sensitivity and data integrity, the workflow should be performed within a controlled laboratory setup, including:

- Physical separation of pre-PCR and post-PCR environments
- Use of dedicated consumables and equipment
- Standardized handling protocols to minimize contamination

9. NGS-based readout and quantification (for in-house implementation only)

The use of NGS as a readout confers several advantages over traditional detection methods, including high sensitivity, broad dynamic range, and scalability, while minimizing signal saturation and enabling precise quantification of low-abundance proteins. Sequencing-based detection also supports efficient multiplexing through sample indexing, allowing large cohorts to be processed in parallel without compromising data quality.

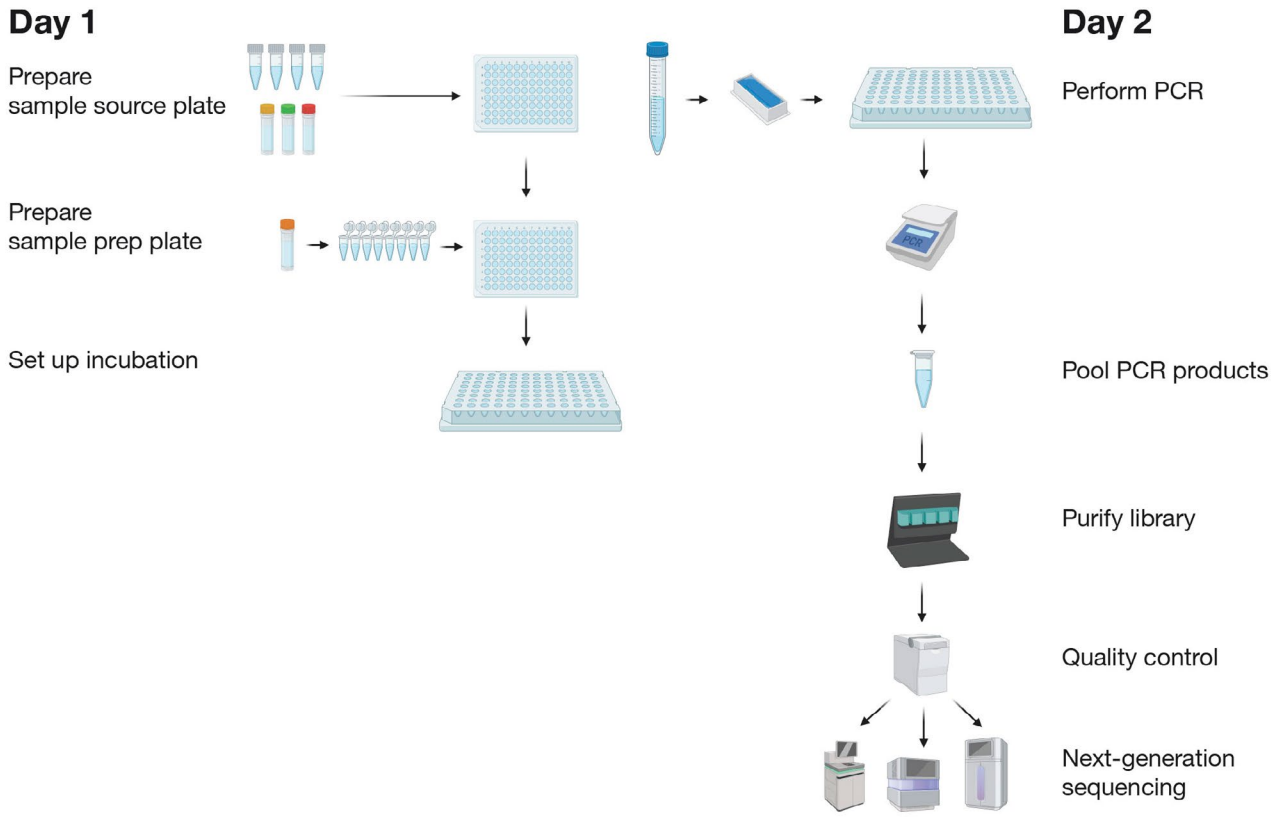

Figure 3: Simplified two-day workflow of the Olink Reveal platform

The platform is compatible with several high-throughput sequencing systems (Illumina NextSeq™ 2000, NovaSeq™ X and NovaSeq 6000, MGI G400, T1+, Element Biosciences AVITI™, and Ultima Genomics systems, ensuring integration with various options of genomic infrastructure.

10. Data generation and normalization (for in-house implementation only)

Protein abundance is quantified using normalized protein expression (NPX) values, generated through a standardized pipeline that includes internal control normalization, log2 transformation, and plate-based normalization. For multi-plates projects, intensity-normalization is also needed.

This structured normalization approach ensures:

- Comparability across samples
- Reduction of technical variation
- Compatibility with downstream statistical and multi-omics analyses

NPX Map: Core data processing, QC, and normalization

Data generated from Olink Reveal are processed using NPX™ Map, a dedicated analysis software designed specifically for Olink platforms. NPX Map enables end-to-end data handling, including data import, quality control (QC), normalization, and export of analysis-ready datasets.

Following sequencing, raw NGS output is converted into count data, representing the number of detected DNA barcodes per protein and sample. These data undergo a structured QC and normalization workflow, including:

- Multi-level QC:
  - Sample QC (e.g., total counts, internal control performance)
  - Assay QC (detection of unexpected signal or background)
  - Block/plate QC (identification of technical failures across runs)
  - Systematic effect detection (e.g., row/column or gradient effects)
- Normalization to NPX (Normalized Protein eXpression):
  - Counts are normalized to internal extension controls
  - Log2 transformation is applied
  - Plate-level normalization corrects inter-run variation

This process generates standardized NPX values, enabling robust comparison across samples, plates, and studies while minimizing technical variability.

NPX Map outputs include:

- NPX and extended NPX datasets
- Quality control summaries
- Analysis reports with QC metrics and metadata

These outputs ensure transparency, traceability, and reproducibility for downstream analysis.

Advanced QC metrics and validation

Beyond standard QC, NPX Map incorporates additional metrics to strengthen data interpretation:

- Olink Sample Index (OSI), which quantifies pre-analytical variation related to sample handling
- Intra- and inter-plate coefficients of variation (CVs), providing quantitative measures of assay precision

Scalable analysis pipelines: NPX Map CLI and preprocessing

For large-scale studies, a command-line interface (NPX Map CLI) is provided to enable automated QC, normalization, and data export within reproducible computational workflows.

Upstream preprocessing tools convert raw sequencing data into count matrices and metadata, ensuring compatibility across sequencing platforms.

These tools support:

- Scalable analysis of large cohorts
- Automation of data processing
- Reproducibility across computational environments

Cross-study and longitudinal comparability (bridging normalization)

While NPX normalization (including internal controls, plate normalization, and intensity normalization) ensures robust comparability within a study, additional strategies are required when combining data across independent runs, batches, or timepoints.

For such cases, Olink solutions support bridging normalization, which uses shared reference samples analyzed across datasets to align protein measurements. By calculating assay-specific adjustment factors based on these overlapping samples, bridging enables direct comparison of NPX values across studies without introducing batch-related bias.

This approach is particularly relevant for:

- Longitudinal studies spanning multiple runs or reagent lots

- Multi-cohort or multi-center studies
- Integration of discovery and validation datasets

When appropriately implemented, bridging normalization ensures that observed differences reflect biological variation rather than technical variability.

11. Downstream analysis and biological interpretation: Olink data ecosystem

11.1 Advanced statistical analysis with Olink™ Analyze

For downstream statistical analysis, Thermo Fisher Olink provides Olink Analyze, a comprehensive R-based framework tailored to proteomic datasets. Olink Analyze is available as a CRAN package.

Olink Analyze enables seamless transition from QC'ed NPX data to advanced statistical modeling:

Data integration and normalization

- Harmonization across plates and studies
- Bridging of datasets using shared samples or reference-based normalization
- Cross-platform integration

Exploratory data analysis

- Principal component analysis (PCA) and UMAP
- Distribution plots and QC visualization
- Detection of outliers and batch effects

Statistical modeling

Supports a wide range of study designs, including:

- Parametric tests (t-tests, ANOVA)
- Non-parametric methods (Wilcoxon, Kruskal–Wallis)
- Linear mixed-effects models for longitudinal data
- Regression models incorporating covariates (e.g. age, sex, clinical variables)

Visualization and interpretation

- Volcano plots and box plots
- Heatmaps and clustering
- Stratified analyses based on covariates or study groups

Biological pathway analysis

- Pathway enrichment (ORA, GSEA)
- Visualization of pathway-level changes
- Integration with multi-omics datasets

This flexibility allows statistical approaches to be tailored to complex experimental designs, including multi-factorial and longitudinal studies.

11.2 Biological interpretation and contextualization: Olink™ Insight

Olink Insight complements statistical analysis by enabling biological interpretation and data contextualization within a unified platform.

Olink Insight is an interactive, online platform that integrates curated datasets, annotation layers, and interactive tools to support the full research workflow:

Study design and planning

- Study size calculator for power estimation
- Assay and pathway selection tools
- Protein list management and export

Pathway and network exploration

- Pathway Browser for mapping proteins to biological pathways
- Automated annotation and enrichment
- Publication Explorer for literature-based context

Biological annotation

- Disease risk associations (e.g., UK Biobank datasets)
- Normal protein expression ranges across large populations
- Tissue specificity and gene ontology
- Proteogenomic associations (pQTLs and heritability)

These features enable differentiation between disease-driven signals and normal biological variation, strengthening biomarker interpretation.

Integration of large-scale datasets

Olink Insight provides access to curated reference datasets (e.g., UK Biobank, China Kadoorie Biobank, Arivale), enabling:

- Cross-study validation
- Hypothesis generation
- Benchmarking against population-scale data

12. Training, technical support, and implementation strategy (for in-house implementation only)

Successful deployment of high-throughput proteomics requires not only robust technology, but also structured training, standardized implementation, and ongoing expert support.

Structured implementation and onboarding workflow

The training process follows a four-phase implementation model, ensuring that laboratories are fully prepared prior to generating study data:

- **Pre-discussions**
  - Alignment on study goals, sample types, and experimental requirements
  - Definition of expectations and success criteria
  - Review of study design considerations (e.g., cohort structure, controls, matrices)
- **Site preparation**
  - Laboratory setup and workflow optimization (pre-PCR/post-PCR separation)
  - Instrument qualification and verification (for new laboratories)
  - Reagent and kit planning
- **Training (hands-on and theoretical)**
  - On-site training by Field Application Scientists (FAS)
  - Combined theoretical instruction and practical workflow training
  - Stepwise onboarding including:
    - Initial test run to practice workflow execution
    - First full run using a combination of control and study samples.
  - Data quality assurance and analytical readiness
    - Standardized QC evaluation using NPX Map
    - Assessment of reproducibility, variability, and concordance
    - Review of results with Olink scientific support

This ensures that:

- The laboratory workflow is performing as expected
- Data quality meets required standards
- Any deviations are identified and corrected early

Ongoing scientific and technical support

Beyond initial onboarding, continuous support is provided throughout the study lifecycle, including:

- Direct access to scientific support teams for troubleshooting and optimization
- Guidance on alternative matrices, sample preparation, and study design
- Support for data analysis workflows, including NPX Map, Olink Analyze, and Olink Insight
- Assistance with scaling studies and increasing throughput
- Planning follow-up studies based on initial findings

This ensures sustained data quality and enables efficient progression from pilot studies to large-scale projects.

13. Alternative Workflow: Olink Analysis Services (AS) Option

Overview of Analysis Services workflow

Olink Analysis Services laboratories (Uppsala, Sweden and Boston, USA) perform analyses using standardized SOP-driven workflows, ensuring high data quality and compliance with applicable regulatory standards.

The workflow with the AS labs includes the following steps:

- Study design and scope of work defined. Study design taking place via the Olink Support and Data Science teams
- Samples are randomized and provided in a 96-well plate format. Samples can be provided as tubes but the sorting, randomizing, and plating adds time and costs
- Data analysis and interpretation in consultation with the Support and Data Science teams

For standard submissions (randomized plates with complete documentation), the contractual honored turnaround time is approximately 4–8 weeks from sample receipt to data delivery. Turnaround time can be shorter or longer based on the complexity of the project, timing of sample arrival, and condition of samples, i.e. all conditions being met to proceed upon receipt

Additional handling steps (e.g., randomization, custom formatting, or special data delivery requirements) may extend timelines and should be considered during study planning.

Advantages of the Analysis Services model

Using Olink Analysis Services provides several advantages:

- No requirement for in-house instrumentation or automation
- Standardized workflows and quality-controlled environment

- Reduced technical variability and operational complexity
- Access to experienced personnel and validated pipelines
- Faster implementation for large-scale or multi-site studies

In addition to Olink Analysis Services, investigators may also access a global network of Olink Certified Service Providers (CSPs). These partners are trained and validated by Olink to deliver high-quality data using PEA technology. A full list of CSPs by region can be found here: https://olink.com/certified-service-providers.

14. Budget Considerations

Implementation of high-throughput proteomics using Olink Reveal involves both platform-specific costs and broader operational expenses associated with laboratory workflows, sequencing, personnel, and data analysis. A comprehensive budget must therefore account for the full end-to-end process, from sample preparation through downstream analysis.

Total cost will depend on:

- Number of samples and study design
- In-house vs. service-based workflow
- Sequencing platform and throughput
- Level of automation and available infrastructure

Consult Thermo Fisher Olink for detailed platform-specific pricing and align internal cost estimates with institutional resources and sequencing facilities.

The Olink Reveal-related costs include:

- Olink Reveal reagent kits (kit-based pricing; each kit supports a defined number of samples)
- Sequencing costs, depending on platform and throughput
- Optional training and onboarding services (basic virtual training included; extended or on-site training available)
- Optional automation support and workflow customization
- Optional data analysis and biostatistical services, including differential analysis and normalization/bridging
- Shipping and cold-chain logistics

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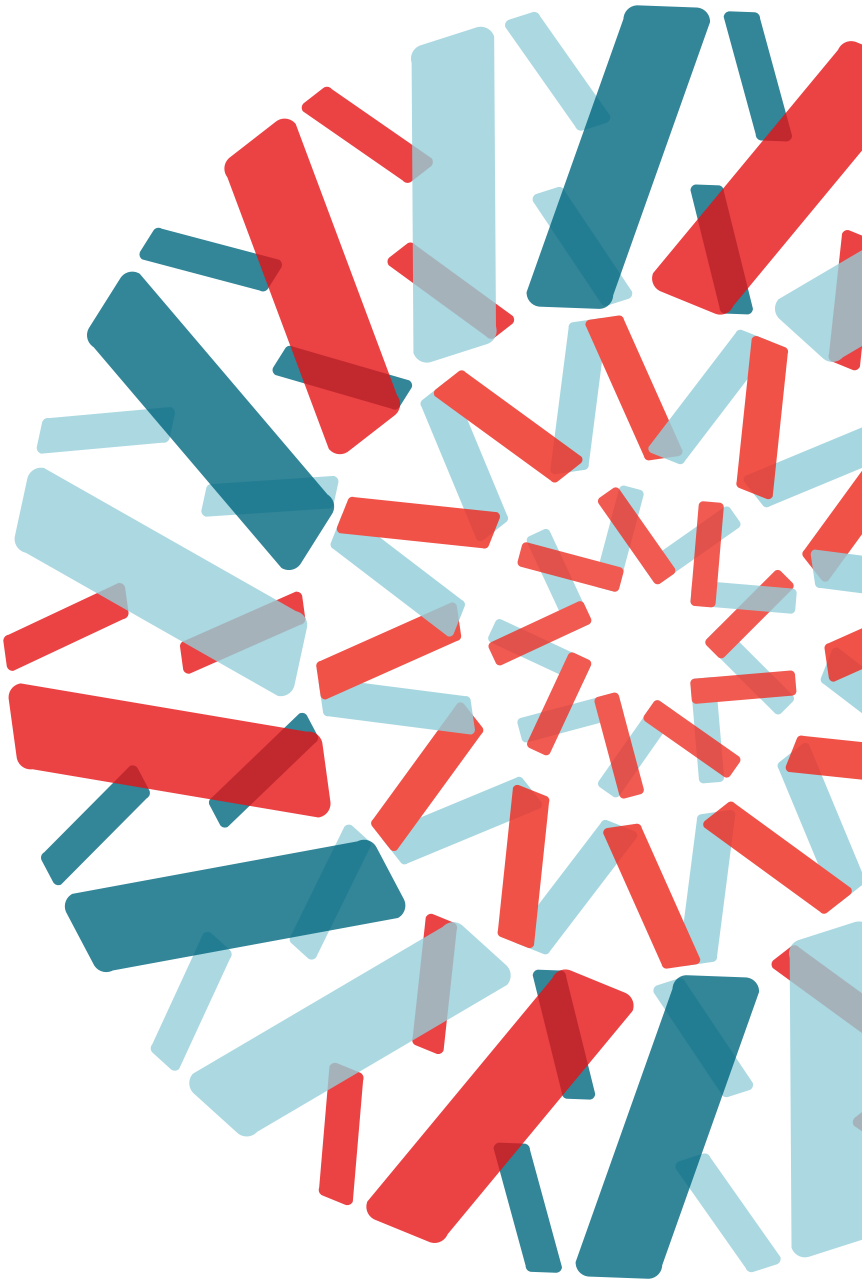

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