# Olink® Reveal Overview

## User Manual

1527, v.1.0, 2025-01-27

# Table of contents

## Part 1: Olink® Reveal overview



## Part 2: How Olink® Reveal works



## Part 3: Site preparation requirements



## Part 4: Guidelines

# 1.Introduction

Circulating plasma proteins could be useful markers for disease prediction, diagnosis, prognosis, and response to treatment. Research into which proteins are regulated by these processes may identify potential biomarkers and drug targets that could help improve the detection and treatment of disease, as well as better understand real-time human biology.

Olink Reveal uses Olink's Proximity Extension Assay (PEA™) technology coupled to next-generation sequencing (NGS). The selected proteins are enriched for immune response and inflammation markers, while broadly covering the proteome according to the Reactome pathways represented. The content is also enriched with protein targets validated as cis pQTLs in independent studies, an orthogonal approach that demonstrates their superior specificity.

The protocol is designed for manual pipetting without the need for specialized equipment, but it could optionally be automated with liquid handling instruments.

## 1.1 Intended use

The product is intended for Research Use Only. Not for use in diagnostic procedures.

Olink® Reveal is designed to measure the relative concentration of approximately 1,000 proteins in human plasma and serum. The laboratory work and data processing shall only be run by staff with appropriate laboratory training. Data processing shall only be performed by trained staff. The results are meant to be used by researchers and should be confirmed by another, independent method.

## 1.2 About this manual

This manual provides an introduction to the Olink® Reveal platform, including information about the reagents, equipment, and documentation needed, an overview of the workflow, as well as laboratory guidelines.

The laboratory and data analysis instructions are not described in this manual, refer to *2. Associated documentation.*

### 1.2.1 Definition of alert levels

The following alert levels are used in the Olink manuals:

Note: Contains information that can make it easier to understand or perform a certain task.

Safe stopping point: Indicates a step where the protocol can be safely paused and restarted at a later time.

# 2.Associated documentation

**User manuals**

- Olink® Reveal Laboratory Instructions
  - Instructions on how to peform the Olink Reveal protocol
- Olink® Reveal Instructions to Automation
  - Guidance on selecting and programing liquid handlers
- NPX™ Map Software User Manual
  - Instructions on how to perform NPX value quality control
- NPX™ Map CLI Technical Information
  - Information about how to integrate data analysis with LIMS and other pipelines
- NPX™ Explore Preprocessing Technical Information
  - Information about the preprocessing with ngs2counts.

**Other documentation**

- List of markers
  - List of proteins targeted and how databases their annotation according to databases
- Validation data
  - List of proteins targeted and their associated validation results
- Safety data sheets (SDS)
  - Documents for each component of the product
- *olink.com/faq/*, search for randomization
  - Describes the importance of sample randomization and provides guidance on how to perform optimal randomization

**Useful tools**

- Study size calculator: *https://insight.olink.com/tools/study-size-calculator*
  - Web-based application to assist in power calculation for a balanced one-way ANOVA
- Stat Analyzer *https://insight.olink.com/tools/stat-analyzer*
  - Web-based application for basic data visualizations and statistical analyses
- Olink Analyze: *https://olink.com/software/olink-analyze*
  - R package designed to streamline the downstream analysis of Olink data, facilitating seamless integration into existing data analysis pipelines. It offers an extensive suite of features, including multi-project normalization, statistical analysis, visualization, and the ability to connect findings to biological databases.

For technical support, contact Olink Proteomics at support@olink.com.

# 3.Principle

There are three core components of Olink Reveal:

- **Pre-mixed, dried-down reagents:** Antibodies and other reagents are pre-mixed and dried into wells of a 96-well plate so that only the samples and controls need to be added to initiate the reaction.
- **Olink® PEA™:** For each protein targeted, two different antibodies raised against the target protein are used. Both antibodies are labeled with unique DNA oligonucleotides. When the antibodies simultaneously bind to their target protein in solution it brings their DNA oligonucleotides into proximity where they hybridize. These are extended and amplified by PCR, resulting in a DNA barcode unique for each protein. Each resulting amplicon also includes specific barcode sequences for each targeted protein, indices for each sample, and adaptors for sequencing.

- **NGS:** Amplified DNA barcodes are pooled from the 96-well plate and purified with magnetic beads. The resulting libraries are loaded onto a validated NGS platform that counts the number of barcodes as a relative measure of the abundance of its associated protein in each sample. These counts are converted into NPX, Normalized Protein eXpression, arbitrary units in the Log2 scale.

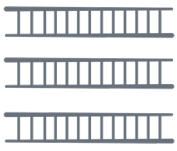
## 3.1 Workflow overview

**Incubation**

Samples are diluted together with controls and transferred to the Reveal Incubation Plate for overnight incubation, during which antibodies bind to their target protein.

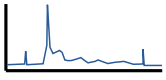
**PCR and pooling**

Oligonucleotides attached to antibodies brought into proximity hybridize, followed by extension and PCR amplification. Unique sample indexes are added to every sample in the amplification step, to enable pooling into a single tube. Each resulting library contains 86 samples and 10 controls that target >1,000 proteins.



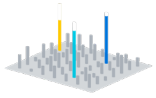
**Library purification and quality control**

The library is purified using magnetic beads. The quality is assessed through an automated electrophoresis platform.



### Sequencing

The library is sequenced using a validated NGS platform. The relative concentration of each targeted protein from each sample is inferred from the counts of its barcode. It is then converted to an NPX value.



### Quality control

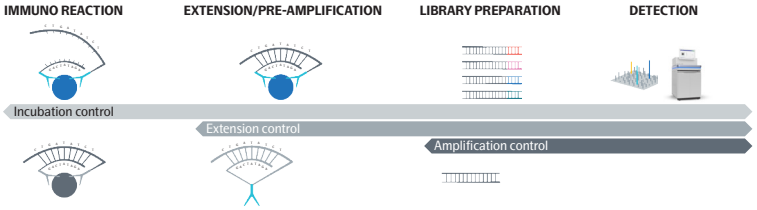
The quality of the run and the resulting NPX values are evaluated using NPX Map desktop or NPX Map CLI software to pinpoint potential sources of errors.

# 4. Quality control

Olink Reveal contains a built-in quality control system using internal and external controls, which enables full control over the technical performance of assays and samples.

## 4.1 Internal controls

Three internal controls are spiked into every sample. The internal controls are designed to monitor the quality of the assay's performance, as well as the quality of individual samples:




**Incubation Control (Immuno Control)**

The Immuno Control is a non-human antigen measured with PEA. This control is included in the immuno reaction and monitors potential technical variation in all three steps of the reaction.

**Extension Control**

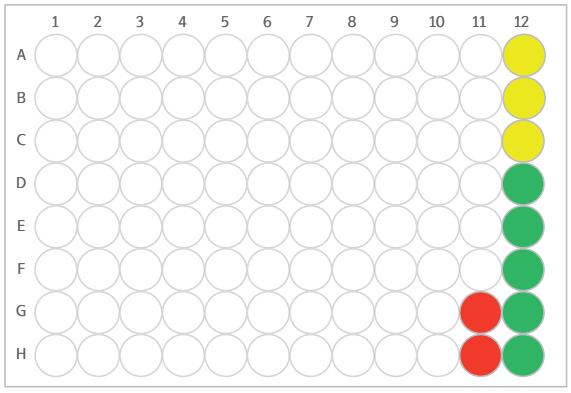
The Extension Control is an antibody coupled to a unique pair of DNA-tags. These DNA-tags are always in proximity so this control is expected to give a constant signal independent of the immuno reaction. This control monitors variation in the extension and amplification step.

**Amplification Control**

The Amplification Control is a double-stranded DNA amplicon that does not require any proximity binding or extension step to generate a signal. This control monitors the amplification/sample indexing step.

## 4.2 External controls

For each plate, 10 external controls are used. These include 3 Sample Controls, 2 Negative Controls, and 5 Plate Controls. They are added according to the plate map below unless they are randomized among samples across the plate.




**Sample Control**

Sample Controls are pooled plasma, with distinct sources from Plate Controls. These are used for troubleshooting and to assess potential variation between runs and plates, for example, to calculate inter-assay and intra-assay CV.

**Plate Control**

Plate Controls are pooled plasma, with distinct sources from Sample Controls. The median of the Plate Controls is used to normalize each assay and compensate for potential variation between runs and plates.

**Negative Control**

Negative Controls are buffers. They are used to monitor any background noise generated when DNA-tags come in close proximity without prior binding to the appropriate protein. The Negative Controls set the background levels for each protein assay and are used to calculate the limit of detection (LOD) and to assess potential contamination of assays.

# 5.General laboratory requirements

The high sensitivity of the Olink assays requires a clean laboratory environment. Particles from the surroundings, such as dust, hair, saliva, and skin flakes are common sources of contamination.

The recommended lab space and storage includes separate Pre-PCR and post-PCR rooms/designated spaces, with separate consumables and equipment.

Freezers and refrigerators are needed to store different reagents at -80 °C , -20 °C , and 4 °C.

## 5.1 Equipment required

| Equipment | Notes |
|---|---|
| Vortex mixer | Recommended 1 each in the pre- and post-PCR rooms |
| Mini centrifuge | |
| Plate centrifuge | |
| Single-channel pipettes | |
| Multichannel pipettes | |
| Timer | |
| Thermal cycler with 96-well block | Heated lid, ensure that 100 µL volume can be used |
| | Required to fit Sarstedt plates, part # 72.1979. A list of compatible thermal cycler models can be found on *https://www.sarstedt.com/* |
| Magnetic rack | |
| Bioanalyzer or TapeStation | Agilent |
| NovaSeq X, NovaSeq X Plus, NextSeq 2000, or NovaSeq 6000 | |

## 5.2 Consumables required

| Consumables | Notes |
|---|---|
| 2x 96-well PCR plates | |
| 2x 8-well strips with caps | |
| Filter pipette tips | |
| Adhesive films | |
| 1x 15 mL tube | |
| 4x 2 mL tubes | |
| Reagent reservoir (25 mL) | |
| AMPure XP beads | |
| 96% ethanol | |
| Milli-Q water | |
| Bioanalyzer High Sensitivity DNA kit or TapeStation D1000 ScreenTape assay | |
| Illumina flowcells that can be run on the sequencer of choice:<br>– 1.5 B Reagent Kit (100 cycles)<br>– 10 B Reagent Kit (50 cycles)<br>– P4 XLEAP-SBS Reagent Kit (50 cycles)<br>– S1 Reagent Kit v1.5 (100 cycles)<br>– S4 Reagent Kit v1.5 (35 cycles) | |

## 5.3 Provided by Olink® Reveal

**For the 1-pack solution**

| Box | Storage temperature | Shipment temperature | Component | Notes | Volume |
|---|---|---|---|---|---|
| 96200A Olink Reveal Plate and PCR Additive | 4 °C | -20 °C | 89003 Olink Reveal PCR Additive | 2.0 mL brown tube and brown lid | 1,520 µL |
| | | | 89002 Olink Reveal Incubation Plate | Contains vacuum dried reagents in each well, wrapped | N/A |
| 96200B Olink Reveal Sample Prep | -20 °C | -20 °C | 89008 Olink Reveal Sample Buffer | 2.0 mL tube with orange lid | 1,375 µL |
| | | | 89001 Olink Reveal PCR Solution | 2.0 mL tube with black lid | 1,520 µL |
| | | | 89009 Olink Explore/Reveal Negative Control | 0.5 mL tube with red lid | 160 µL |
| | | | 89006 Olink Reveal Enzyme A | 1.5 mL tube with blue lid, looped to the tube | 320 µL |
| | | | 89007 Olink Reveal Enzyme B | 1.5 mL tube with purple lid, looped to the tube | 50 µL |
| 96200C Olink Reveal Controls | -80 °C | -80 °C | 89011 Olink Explore/Reveal Sample Control | 0.5 mL tube with yellow lid | 95 µL |
| | | | 89010 Olink Explore/Reveal Plate Control | 0.5 mL tube with green lid | 160 µL |

**For the 10-pack solution, used when large orders are placed to reduce packaging waste**

| Box | Storage temperature | Shipment temperature | Component | Notes | Volume |
|---|---|---|---|---|---|
| 96200D Olink Reveal PCR Additive, 10p | 4 °C | -20 °C | 89003 Olink Reveal PCR Additive x10 | 2.0 mL brown tube and brown lid | 1,520 µL |
| 96200E Olink Reveal Plate 10p | 4 °C | -20 °C | 89002 Olink Reveal Incubation Plate x10 | Contains vacuum dried reagents in each well, wrapped | N/A |
| 96200F Olink Reveal Sample Prep, 10p | -20 C | -20 °C | 89008 Olink Reveal Sample Buffer x10 | 2.0 mL tube with orange lid | 1,375 µL |
| | | | 89001 Olink Reveal PCR Solution x10 | 2.0 mL tube with black lid | 1,520 µL |
| | | | 89009 Olink Explore/Reveal Negative Control x10 | 0.5 mL tube with red lid | 160 µL |
| | | | 89006 Olink Reveal Enzyme A x10 | 1.5 mL tube with blue lid, looped to the tube | 320 µL |
| | | | 89007 Olink Reveal Enzyme B x10 | 1.5 mL tube with purple lid, looped to the tube | 50 µL |
| 96200G Olink Reveal Controls, 10p | -80 °C | -80 °C | 89011 Olink Explore/Reveal Sample Control x10 | 0.5 mL tube with yellow lid | 95 µL |
| | | | 89010 Olink Explore/Reveal Plate Control x10 | 0.5 mL tube with green lid | 160 µL |

# 6.Software

The following must be installed to support sequencing and quality control.

- Custom recipe
  - Installed on the NGS instrument to give instructions on how to sequence Olink Reveal library. Provided by Olink Support.
- Preprocessing software (ngs2counts)
  - Installed on the local computer for the generation of counts files for completed runs. For more information, refer to the NPX™ Explore Preprocessing Technical Information.
- NPX™ Map software
  - Installed on the local computer for the analysis of counts files and quality control for completed runs. For more information, refer to the NPX™ Map Software User Manual.
- NPX™ Map CLI software
  - Optional installation. The CLI version of the desktop software provides a command-line interface for executing the same functions and operations, enabling streamlined automation and integration into customized workflows.

# 7.Safety considerations

## 7.1 Safety instructions

Follow general laboratory safety procedures:

- Use gloves, safety goggles, and protective clothing when performing the experiments.
- Handle solutions with particular caution, as Dimethyl sulfoxide (DMSO) is known to facilitate the entry of organic molecules into tissues.
- Handle and dispose of hazardous sample material according to local regulations.

For complete safety information, refer to Safety Data Sheets (SDS) available on the Olink website: *https://olink.com/knowledge/documents*.

## 7.2 Clean laboratory environment

The high sensitivity of the Olink Reveal assays requires a clean laboratory environment. Particles from the surroundings, such as dust, hair, saliva, and skin flakes are common sources of contamination. The following recommendations are intended to reduce the risk of contamination, simplify the workflow in the laboratory, and improve data quality.

- Use separate rooms for pre-PCR and post-PCR operations.
- Use separate consumables and equipment for pre-PCR and post-PCR operations.
- Always work from clean areas free from PCR products (pre-PCR) to areas containing PCR products (post-PCR).
- Fit ultra-violet (UV) lamps in closed working areas such as working cabinets or pipetting robots to enable decontamination by irradiation.
- Always wear a long-sleeved lab coat.
- Always wear gloves, including when bringing reagents in and out of the fridge or freezer. Change gloves when needed.
- Wash your hands and change gloves and lab coat when moving between pre-PCR and post-PCR.
- Regularly decontaminate bench spaces with 10 % sodium hypochlorite (followed by water to remove residual bleach), or a validated commercially available DNA-degrading decontaminant.
- Decontaminate pipettes regularly according to the manufacturer's instructions.
- Clean instruments and pipetting robots regularly according to the manufacturer's instructions.
- Keep all consumables (tubes, pipette tips, PCR plates, etc.) in closed bags or boxes, preferably in a closed storage unit, until use.
- All consumables and reagents are for single use only.
- Clean the lab bench, hood, racks, and pipettes with 70 % ethanol.
- Bring out all reagents, consumables, and samples needed for the specific lab step at the beginning of each instruction. Leave enzymes in the freezer until use.
- Organize equipment, consumables, and samples at the workstation in a way that enables clean work.
- Label pipette boxes with column numbers to more easily monitor where you are on the plate.
- Briefly centrifuge tubes and plates before opening to avoid the generation of aerosols that may contaminate other samples.
- Pipette all reagents and samples using filter tips, and use a unique set of pipettes for each working station.

## 7.3 Temperatures

Unless otherwise stated in the protocol, all steps should be completed at room temperature, defined as 18–27 °C.

# 8.Randomizing samples

Proper sample randomization improves the power of a study and ensures that technical variation is not confounded with biological variation. When a study is well randomized the experimental variables can be considered to be evenly distributed across each plate, as well as between the plates of a larger study. For most studies, samples can be completely randomized across the plates. In the case of longitudinal studies, we recommend keeping samples from the same subject on the same plate to preserve the paired nature of the study and minimize technical variability between paired samples.

Olink applies Intensity normalization to the final NPX data as default for all studies where samples are randomized. If samples cannot be properly randomized intensity normalization should not be applied to your data set and alternative normalization needs to be applied. For optimal normalization of your study please refer to *Olink FAQ*.

## 8.1 How to randomize your samples

The simplest way to randomize your samples is to use a random number generator. Assign a random number to all your samples, sort them according to the random numbers, and place the samples accordingly in your plate or sort your tubes in the given order.

For more information on plate randomization and instructions on how to generate and evaluate randomized plates, Olink Analyze offers a tutorial on plate randomization (*https://cran.r-project.org/web/packages/OlinkAnalyze/vignettes/plate_randomizer.html*).

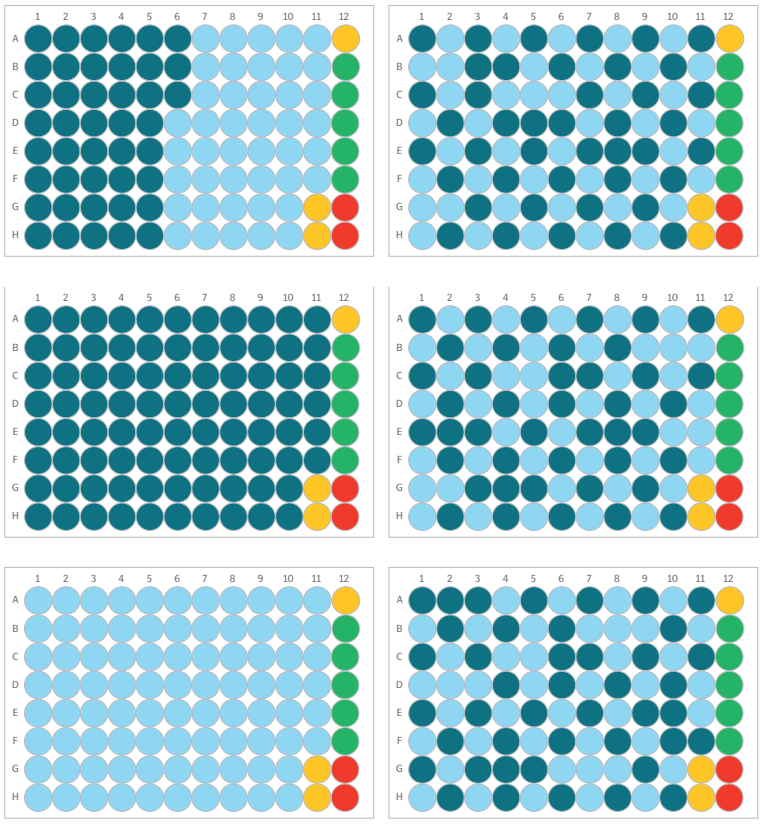
For questions or further recommendations on how to randomize your project, please contact support at support@olink.com..

**Optimal randomization of one plate of samples:**
For studies with one plate of samples or less (n ≤88), randomization of the samples will make the quality control process of the data more robust as technical variation can be more easily identified.

Controls are in columns 11–1, as shown in figures on next page.

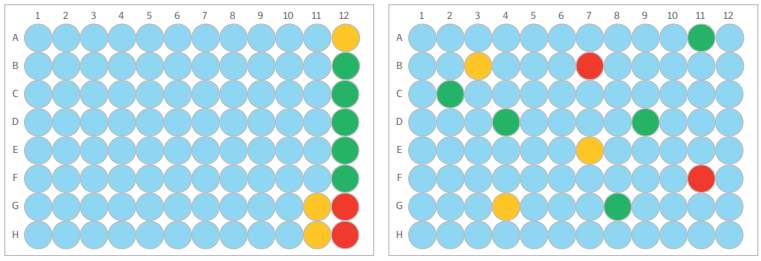
**Optimal randomization of studies with more than one plate of samples**
For studies with more than one plate of samples (n ≥89) unequal distribution of the samples across plates can remove true biological variation following normalization.

Controls are in columns 11–1, as shown in figures on next page.



# 9.Randomizing external controls

Although the default is to use the standard positions for external controls for all experiments, some users may choose to also randomize the external controls among the samples in the plate, then later instruct NPX Map and NPX Map CLI where the positions for each control is, for each plate being analyzed. This enables NPX Map and NPX Map CLI to indicate if there are plate labeling errors, such as if a plate swap has occurred during the experiment.



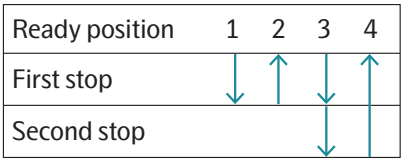
# 10. Pipetting techniques

## 10.1 General pipetting guidelines

- Calibrate all pipettes at least every 6 months.
- Pipette near the liquid surface, except when pipetting into the Reveal Incubation Plate, where the pellet or liquid surface should not be touched.
- Do not turn the pipette on the side when there is liquid in the tip, as liquid might contaminate the interior of the pipette.
- If using a multichannel pipette, ensure that all tips contain the exact same volume.

## 10.2 Forward pipetting

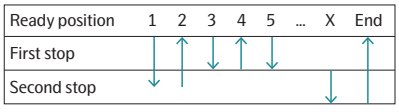
1. Press the operating button to the first stop.
2. Dip the tip into the solution to a depth in accordance with the set volume, and slowly release the operating button. Remove the tip from the liquid.
3. Dispense the liquid into the receiving vessel by gently pressing the operating button to the first stop and then to the second stop. This action will empty the tip. Remove the tip from the vessel.
4. Release the operating button to the ready position.




## 10.3 Reverse pipetting

When using reverse pipetting, pre-rinse the tip 1 to 3 times with the liquid to be pipetted to improve accuracy.

1. Press the operating button past the first stop.
2. Dip the tip into the solution to a depth in accordance with the set volume, and slowly release the operating button. This action will fill the tip with a volume that is larger than the set volume.
3. Remove the tip from the liquid and dispense the liquid into the receiving vessel by pressing the operating button gently and steadily to the first stop. The dispensed volume is equal to the set volume.
4. Hold the button in this position. Some liquid will remain in the tip, which should not be dispensed.
5. Continue pipetting by repeating steps 3 and 4.




# 11. Vortexing

For tubes, a manual vortex is needed to vortex all separate tubes throughout the protocol.
For plates, set the vortex at full speed and ensure that there is a swirling motion in all wells.

DO NOT use a microplate vortexer like a MixMate.

# 12. Revision history

| Version | Date | Description |
|---|---|---|
| 1.0 | 2025-01-27 | New |

www.olink.com



1527, v.1.0, 2025-01-27