



Olink[®] Explore HT Sequencing using NovaSeq[™] X Plus

User Manual

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Part 1: Overview

1. Introduction

1.1 Intended use

Olink® Explore HT is a multiplex immunoassay platform for human protein biomarker discovery. The product is intended for Research Use Only, and not for use in diagnostic procedures. The laboratory work shall only be run by trained laboratory staff. Data processing shall only be performed by trained staff. The results are meant to be used by researchers in conjunction with other clinical or laboratory findings.

1.2 About this manual

This manual provides the instructions needed to sequence Olink® Explore HT libraries on Illumina® NovaSeq™ X Plus using the NovaSeq™ X Series 10B Reagent Kit (100 Cycle). For full instructions on how to perform a sequencing run, refer to the manufacturer's instructions.

The instructions described in this manual must be strictly and explicitly followed. Any deviations throughout the laboratory steps may result in impaired data.

For instructions on how to run the Olink® Explore HT Reagent Kits, refer to the Olink® Explore HT User Manual.

For data processing and analysis of the Olink® Explore sequence results, refer to the NPX™ Explore HT & 3072 User Manual.


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1.2.1 Definition of alert levels


The following alert levels are used in the Olink user manuals:

 **WARNING:** Indicates a potentially hazardous situation which, if not avoided, could result in injury.

 **IMPORTANT:** Indicates an important action that may impair the results if not performed correctly.

 **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.

 **TIME SENSITIVE STEP:** Indicates a step that must be performed within a limited time period. Results may be impaired if not performed correctly.

2. Associated documentation

2.1 Olink documentation

- Olink® Explore HT User Manual
- NPX™ Explore HT & 3072 User Manual

2.2 Other documentation

For information on the Illumina Novaseq X Plus, read the following technical information that can be found on www.illumina.com:

- Technical information for using the NovaSeq X Plus Sequencing System, Doc no 200027529

3. Technical support

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

Part 2: Laboratory instructions

4. Plan the sequencing run

One Olink® Explore HT Library can be sequenced per NovaSeq X 10B flow cell. After pooling and dilution/denaturation, the library is loaded on all 8 lanes of the flow cell.

When sequencing individual (not pooled) Olink Explore HT blocks, load each block on 2 consecutive lanes on the flow cell (lane 1+2, lane 3+4, lane 5+6, or lane 7+8). This approach requires two flow cells to complete sequencing of all eight blocks and is therefore only recommended for resequencing.

5. Install Olink® custom recipe

Install the applicable custom recipe on the NovaSeq X according to manufacturer's instruction and make sure to use the correct custom recipe for your planned sequencing run.

Library	Flow cell	Custom recipe	NovaSeq X control software	Cycle numbers
Olink Explore HT, standard	10B	Olink_NovaSeqX_10B_1Lib_V2	v1.2	24-0-0-0
Olink Explore HT, individual blocks	10B	Olink_NovaSeqX_10B_4Lib_V2	v1.2	24-0-0-0

6. Dilute Olink® Libraries

Olink recommends using a set library dilution of 1:250. The instructions below replace the instructions for library dilution in the manufacturer's instructions:

Prepare bench


- PL/Lib Tube, prepared according to the Olink Explore HT User Manual.
- Illumina Resuspension Buffer (RSB) or nuclease free water
- 1x Microcentrifuge tubes (1.5 mL)
- Manual pipettes (0.5–10 µL, 100–1000 µL)
- Filter pipette tips


Before you start

- Thaw the PL/Lib tube if frozen.
- Mark the new microcentrifuge tube as "Dil"

Instructions

1. Add 1245 µL of RSB into the Dil Tube.
2. Vortex the PL/Lib Tube and spin down briefly.
3. Transfer 5 µL from the PL/Lib Tube to the Dil Tube.
4. Vortex the Dil Tube and spin down briefly.
5. Use 34 µL of the diluted library for each denaturing reaction.

 **NOTE:** One denaturing reaction is needed per lane to be loaded. When loading the same library on multiple lanes, the denaturing reaction can be scaled up accordingly.

 **NOTE:** Store the PL/Lib Tube(s) at -20 °C in case of potential rerun(s).

Proceed according to manufacturer's instructions for denaturing the library, loading reagents, and performing the sequencing run. For more information, refer to [2.2 Other documentation](#).

7. Revision history

Version	Date	Description
1.0	2024-03-07	New

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