



Olink[®] Explore Sequencing using NextSeq[™] 550

User Manual

Document note

The Olink® Explore User manual, doc nr 1153, is obsolete, and has been replaced by the following documents:

- Olink® Explore Overview User Manual, doc nr 1187
- Olink® Explore 384 User Manual, doc nr 1188
- Olink® Explore 4 x 384 User Manual, doc nr 1189
- Olink® Explore 1536 & Expansion User Manual, doc nr 1190
- Olink® Explore 3072 User Manual, doc nr 1191
- Olink® Explore Sequencing using NextSeq 550 User Manual, doc nr 1192
- Olink® Explore Sequencing using NextSeq 2000 User Manual, doc nr 1193
- Olink® Explore Sequencing using NovaSeq 6000 User Manual, doc nr 1194

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1. Introduction

1.1 Intended use

Olink® Explore 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 User Manual provides the instructions needed to sequence Olink® Explore Libraries on Illumina® NextSeq™ 550. The instructions must be strictly and explicitly followed. Any deviations throughout the laboratory steps may result in impaired data.

Prior to starting the laboratory workflow, consult the Olink® Explore Overview User Manual for an introduction to the platform, including information about reagents, equipment and documentation needed, an overview of the workflow, as well as laboratory guidelines.

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

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

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1.3 Technical support

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

2. Laboratory instructions

This chapter provides instructions on how to sequence Olink Libraries on NextSeq™ 550 using the NextSeq™ 500/550 High Output Kit v2.5 (75 Cycles). The protocol used for sequencing is an adaptation of the Illumina® standard NGS workflow for Illumina® NextSeq™ 550.

Before proceeding to sequencing, make sure that the quality of the purified Library has been verified. Refer to the applicable Olink Explore User Manual for instructions about quality control.

2.1 Plan the sequencing run

One Olink Library can be sequenced per NextSeq™ 550 High Output flow cell and per run. The number of High Output flow cells and runs required to sequence the different Olink Explore Reagent Kits is described in [Table 1](#).

If more than one run is required, repeat the instructions described in this manual.

Table 1. Sequencing run planning

Olink® Explore Reagent Kit	Number of Olink Libraries	Number of flow cell(s) and run(s)
Olink® Explore 384 Reagent Kit	1	1
Olink® Explore 4 x 384 Reagent Kit	4	4
Olink® Explore 1536 Reagent Kit	4	4
Olink® Explore Expansion Reagent Kit	4	4
Olink® Explore 3072 Reagent Kit	8	8

2.2 Install Olink® custom recipe

During this step, the Olink® custom recipe is installed on the NextSeq™ 550. This step only needs to be performed once, before an Olink sequencing run is performed for the first time.

 **NOTE:** The Olink custom recipe will only work with the NextSeq™ 500/550 High Output Kits and the NextSeq™ Control Software 4.0.

1. Unzip and place the Olink custom recipe *Olink_NSQ550_HighOutput_V1* in the following folder of the NextSeq™ 550 instrument: C:\Program Files\Illumina\NextSeq Control Software\Recipe\Custom\High\.
2. Under *System Customization > Manage Instrument*, enable *Custom Recipes*. If not selected, the custom recipe option will not appear during the run setup.

 **NOTE:** In the NCS 4.0 software version, the option to select a custom recipe will only occur after the reagent cartridge is loaded, not in the earlier setup page.

 **NOTE:** The run must be set in manual mode to allow custom recipes.

2.3 Prepare sequencing reagents

During this step, the reagent cartridge containing clustering and sequencing reagents is thawed and the flow cell is prepared.

2.3.1 Prepare reagent cartridge



WARNING: The reagent cartridge contains potentially hazardous chemicals. Wear adequate protective equipment and discard used reagents in accordance with applicable standards. For more information, refer to the Illumina NextSeq 550 System Guide (document #15069765).

Prepare bench

- 1x NextSeq™ 500/550 High Output Reagent Cartridge v2 (75 cycles).

Instructions

1. Place the frozen reagent cartridge half-submerged in room tempered water and let it thaw for 1 hour. Make sure that all reagent reservoirs of the cartridges are completely thawed.



NOTE: For convenience, thaw the cartridge the day before and store it over night at 4 °C. At this temperature, reagents are stable up to one week.

2. Dry the cartridge base thoroughly with a paper towel and blot the foil seals dry with a lint-free tissue if needed.
3. Invert the cartridge ten times to thoroughly mix the thawed reagents within.
4. Tap the cartridge gently on the bench to remove air bubbles. Store the cartridge at room temperature if it will be used within 4 hours.

2.3.2 Prepare flow cell

Prepare bench

- 1x NextSeq™ 500/550 High Output Flow Cell v2.5.

Instructions

1. Bring the refrigerated flow cell to room temperature for 30 minutes.
2. Put on new powder free gloves (to avoid contaminating the glass surface of the flow cell).
3. When ready to load the flow cell into the instrument, remove the flow cell from the package and the plastic clamshell.
4. Inspect the flow cell. If particulate or dust is visible on any of the glass surfaces, clean the applicable surface with a lint-free isopropyl alcohol wipe and dry it with a low-lint lab tissue.

2.4 Prepare Olink® Library for sequencing

During this step, NaOH and Tris-HCl dilutions are prepared, and the purified and quality controlled Olink Library is diluted and denatured in sequential steps.

2.4.1 Prepare NaOH dilution

The NaOH dilution is used to denature the Libraries.

Prepare bench

- 1 N NaOH stock
- MilliQ water
- 1x Microcentrifuge tube (1.5 mL)
- Manual pipette (10–100 µL)
- Filter pipette tips

Before you start

Mark the microcentrifuge tube “0.2 N NaOH”.

Instructions

5. Prepare a 0.2 N NaOH dilution in the 0.2 N NaOH Tube according to [Table 2](#).
6. Vortex the 0.2 N NaOH Tube thoroughly and spin down. Use within 12 hours.

Table 2. 0.2 N NaOH dilution

Reagent	Volume (µL)
MilliQ water	80
1 N NaOH stock	20

2.4.2 Prepare Tris-HCl dilution

The Tris-HCl dilution is used to neutralize the denaturated Library.

Prepare bench

- 1 M Tris-HCl pH 7.0 stock (Trizma® hydrochloride solution)
- MilliQ water
- 1x Microcentrifuge tube (1.5 mL)
- Manual pipette (10–100 µL)
- Filter pipette tips

Before you start

- Mark the microcentrifuge tube “Tris-HCl”

Instructions

1. Prepare a 200 mM Tris-HCl dilution in the Tris-HCl Tube according to [Table 3](#).
2. Vortex the Tris-HCl Tube thoroughly and spin it down.

Table 3. 200 mM Tris-HCl dilution

Reagent	Volume (µL)
MilliQ water	80
1M Tris-HCl pH 7.0 stock (Trizma® hydrochloride solution)	20

2.4.3 Dilute Olink® Libraries

During this step, the purified and quality controlled Olink Library is diluted 1:33.

Prepare bench

- Lib Tube, prepared according to the applicable Olink Explore User Manual
- MilliQ water
- 1x Microcentrifuge tube (1.5 mL)
- Manual pipettes (0.5–10 and 100–1000 µL)
- Filter pipette tips

Before you start

- Thaw the Lib Tube if frozen.
- Mark the new microcentrifuge tube: “Dil”.

Instructions

1. Add 96 μL of MilliQ water to the Dil Tube.
2. Vortex the Lib Tube and spin it down briefly.
3. Transfer 3 μL from the Lib Tube to the Dil Tube.
4. Vortex the Dil Tube and spin it down briefly.



NOTE: Store the Lib Tube(s) at $-20\text{ }^{\circ}\text{C}$ in case of potential rerun(s).

2.4.4 Denature and dilute Olink® Library to the final loading concentration

During this step, the diluted Olink Library is denatured and further diluted to the final loading concentration.

Prepare bench

- Dil Tube, prepared in previous step
- 0.2 N NaOH dilution, freshly prepared in previous step
- 200 mM Tris-HCl (pH 7.0) dilution, prepared in previous step
- Hybridization buffer 1 (HT1) included in the NextSeq™ Accessory Box v2
- 2x Microcentrifuge tubes (1.5 mL and 2 mL)
- Manual pipettes (0.5–10 and 100–1000 μL)
- Filter pipette tips

Before you start

- Thaw the frozen HT1 buffer at room temperature. Store at $+4\text{ }^{\circ}\text{C}$ until use.
- Mark the new 1.5 mL microcentrifuge tube: "Den" (for the denatured Library).
- Mark the new 2 mL microcentrifuge tube: "Seq" (for the ready to load Library).

Instructions

1. Transfer 5 μL from the Dil Tube to the Den Tube.
2. Add 5 μL of 0.2 N NaOH to the Den Tube.
3. Vortex the Den Tube and spin it down briefly.
4. Incubate the Den Tube for 5 minutes at room temperature to denature the Library.
5. Add 5 μL of 200 mM Tris-HCl (pH 7.0) to the Den Tube to neutralize the reaction.
6. Vortex the Den Tube and spin it down briefly.
7. Add 985 μL of prechilled HT1 to the Den Tube.
8. Vortex the Den Tube and spin it down briefly. The tube can be stored at $+4\text{ }^{\circ}\text{C}$ until use (the same day).
9. Transfer 205 μL from the Den Tube to the Seq Tube.
10. Add 1095 μL of prechilled HT1 to the Seq Tube.
11. Invert the Seq Tube to mix the reagents and spin it down briefly. The final loading volume is 1.3 mL.
12. Immediately continue to [2.5 Perform Olink® sequencing run](#).

2.5 Perform Olink® sequencing run

During this step, the buffer cartridge, the flow cell and the prepared reagent cartridge containing the Olink Library are loaded into the NextSeq 550, and the sequencing run is started using the Olink custom recipe.

Prepare bench

- Seq Tube (with ready to load Library), prepared in previous step
- 1x NextSeq™ 500/550 High Output Reagent Cartridge v2, prepared in previous step
- 1x NextSeq™ 500/550 High Output Flow Cell v2.5, prepared in previous step
- 1x NextSeq™ 500/550 Buffer Cartridge v2 (75 cycles), at room temperature

2.5.1 Set up sequencing run parameters

During this step, sequencing run parameters are selected on the NextSeq™ 550.

1. On the NextSeq™ 550 *Home* screen, select *Experiment*.
2. On the *Select Assay* screen, select *Sequence*.
3. In the *Run Setup* page, select *Manual run mode* and then *Next*.
4. Set up the run parameters as follows:
 - In the *Run Name* field, enter a unique experiment ID.
 - In the *Library ID* field, enter the ID of the Library you are running (optional).
 - In the *Read Type* field, select the *Single Read* option.
 - Enter the number of cycles as follows:
 - o Read 1: 24
 - o Index 1: 0
 - o Index 2: 0
 - o Read 2: 0

 **IMPORTANT:** *It is crucial that Read 1 is set to 24, otherwise the entire run will fail.*

- Keep the checkbox for custom primers unselected.
 - Set the output folder location for the current run raw data. Select *Browse* to change the output folder location.
5. Do not set up a Sample Sheet.
 6. Select *Purge consumables for this run*.
 7. Select *Next*.

2.5.2 Load flow cell into NextSeq™ 550

1. Remove the used flow cell from the previous run.
2. Place the new prepared flow cell on the stage.
3. Select *Load*. The door is automatically closed.
4. When the flow cell ID appears on the screen and the sensors are checked in green, select *Next*.

2.5.3 Empty the reagent container

 **WARNING:** **This set of reagents contains potentially hazardous chemicals. Wear adequate protective equipment and discard used reagents in accordance with applicable standards. For more information, refer to the Illumina NextSeq 550 System Guide.**

1. Open the door of the buffer compartment, remove the spent reagents container from the lower compartment, and dispose of the content in accordance with applicable standards.
2. Slide the empty reagent container back into the lower buffer compartment. An audible click indicates that the container is correctly placed.

2.5.4 Load buffer cartridge

1. Remove the used buffer cartridge from the upper buffer compartment and dispose of the content in accordance with applicable standards.
2. Slide a new buffer cartridge into the upper buffer compartment. An audible click indicates that the cartridge is correctly placed. Make sure that the buffer cartridge ID appears on the screen and that the sensors are checked in green.
3. Close the door of the buffer compartment and select *Next*.

2.5.5 Load reagent cartridge

1. Open the door of the reagent compartment, remove the used reagent cartridge, and dispose of the unused content in accordance with applicable standards. The reservoir at position 6 is removable to facilitate safe disposal.
2. Pierce the seal of the reservoir #10 labeled as “Load Library Here” with a clean 1 mL pipette tip.
3. Load 1.3 mL the Olink Library from the Seq Tube into reservoir #10 labeled as “Load Library Here”.
4. Slide the new reagent cartridge into the reagent compartment and close the reagent compartment door.
5. Select *Load* and wait for ~30 seconds until the reagent cartridge ID appears on the screen and the sensors are checked in green.
6. From the *Recipe* drop-down list, select the [Custom] “Olink_NSQ550_HighOutput_V1” recipe option.

 **IMPORTANT:** Make sure that the custom recipe was previously installed in the instrument. Refer to [2.2 Install Olink® custom recipe](#).

7. Select *Next*.

2.5.6 Start sequencing run

1. Confirm the run parameters displayed on the *Review* screen. To edit any parameters, press *Back* to return to the *Run Setup* screen.
2. Select *Next*. The run starts after an automatic pre-run check. The sequencing run time is approximately 7h30 min.

 **IMPORTANT:** Make sure that the run starts when the automatic pre-run check has been completed (~5 minutes).

 **NOTE:** For any pre-run check failures, refer to the manufacturer's instructions.

 **NOTE:** Be careful not to bump into or otherwise disturb the NextSeq™ 550 during the sequencing run. The instrument is sensitive to vibrations.

3. Clean the work area.

 **NOTE:** When the sequencing run is completed, the software initiates an automatic post-run wash using the wash solutions provided in the buffer cartridge and the NaOCl provided in the reagent cartridge. This wash takes approximately 90 minutes. The *Home* button becomes active once the wash is completed. Used cartridges and flow cell can be left in place until the next run.

2.5.7 Monitor run progress

Olink uses NGS as readout to quantify the amount of a known sequence in order to estimate the concentration of a given protein in samples (relative to other samples). Data quality from each Explore sequencing run is mainly determined by QC parameters unique to Olink technology. Hence, standard quality control metrics used in conventional NGS, such as Q-score, are less critical.

3. Revision history

Version	Date	Description
1.2	2022-11-01	1.2 Replaced Olink® Mydata with Olink® NPX Explore. 2.4 Added 0.2 N to Before you start.
1.1	2021-12-13	Editorial changes
1.0	2021-12-01	New

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