



Olink[®] Explore Sequencing using NextSeq[™] 2000

User Manual

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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™ 2000. 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 applicable Olink® NPX Explore software 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™ 2000 using the NextSeq™ 1000/2000 P2 Reagents (100 Cycles) v3. The protocol used for sequencing is an adaptation of the Illumina® standard NGS workflow for Illumina® NextSeq™ 2000.

Before proceeding to sequencing, make sure that the quality of the purified Olink 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™ 2000 P2 flow cell and per run. The number of P2 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 3072 Reagent Kit	8	8

2.2 Prepare Olink® Custom recipe

During this step, the Olink® custom recipe is prepared on the NextSeq™ 2000. This step only needs to be performed once, before an Olink sequencing run is performed for the first time.

Unzip and save the Olink custom recipe xml-file Olink_NSQ2K_P2_V1 in an appropriate instrument folder.



NOTE: The Olink custom recipe will only work with the NextSeq™ 1000/2000 P2 Reagents (100 Cycles) v3 kit and the NextSeq™ 1000/2000 control software v1.2 or v1.4.

2.3 Prepare sequencing reagents

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



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 1000 and 2000 System Guide (document #1000000109376).

2.3.1 Prepare reagent cartridge

Thawing the unopened cartridge can be done using three different methods: at room temperature, in a controlled water bath, or in the refrigerator.

Prepare bench


- 1x NextSeq™ 1000/2000 P2 Reagent Cartridge (100 cycles)

Instructions

1. Thaw the reagent cartridge as described in [Table 2](#).

Table 2. Reagent cartridge thawing methods

Thawing method	Instructions
At room temperature	<ul style="list-style-type: none"> • Without opening the silver foil bag, place the frozen reagent cartridge on the bench and thaw it at room temperature for 9 hours. Do not exceed 16 hours. • Make sure that the bag label faces up and that air can circulate around the cartridge.
In a water bath	<ul style="list-style-type: none"> • Without opening the silver foil bag, place the frozen reagent cartridge half-submerged in a controlled 25 °C water bath and let it thaw for 6 hours. Do not exceed 8 hours. • Make sure that the bag label faces up and that the cartridge does not invert during thawing. • Dry the cartridge thoroughly with a paper towel.
In the refrigerator	<ul style="list-style-type: none"> • Without opening the silver foil bag, place the cartridge on the bench and thaw it at room temperature for 6 hours. • Make sure that the bag label faces up and that air can circulate around the cartridge. • Finish thawing the cartridge in the refrigerator for 12 hours. Do not exceed 72 hours. • Before the run, remove the thawed unopened cartridge from the refrigerator and allow it to reach room temperature during at least 15 minutes, but not more than 1 hour.

 **NOTE:** Thawed cartridges cannot be refrozen and must be stored at 4 °C, for a maximum time of 72 hours.

2.3.2 Prepare flow cell

Prepare bench

- 1x NextSeq™ 1000/2000 P2 Flow Cell

Instructions

1. Bring the refrigerated flow cell to room temperature for 10-15 minutes.

2.4 Prepare Olink® Library for sequencing

During this step, the purified and quality controlled Olink Library is diluted to the final loading concentration. Note that Library denaturation is performed automatically onboard the instrument.

Prepare bench

- Lib Tube, prepared according to the applicable Olink Explore User Manual
- 1x RSB with Tween 20
- MilliQ water
- 2x Microcentrifuge tubes (1.5 mL)
- Manual pipette (10, 100 and 1000 µL)
- Filter pipette tips

Before you start

- Thaw the Lib Tube if frozen.
- Thaw the frozen RSB with Tween 20 at room temperature for 10 minutes. Store at +4 °C until use.
- Mark the two new 1.5 mL microcentrifuge tubes as follows:
 - Mark one tube "Dil" (for the 1:100 diluted Library)
 - Mark one tube "Seq" (for the ready to load Library)

Instructions

1. Add 495 µL of MilliQ water to the Dil Tube.
2. Vortex the Lib Tube and spin it down briefly.
3. Manually transfer 5 µL from the Lib Tube to the Dil Tube.
4. Vortex the Dil Tube and spin it down briefly.
5. Add 20 µL of RBS with Tween 20 to the Seq Tube.
6. Manually transfer 20 µL from the Dil Tube to the Seq Tube.
7. Vortex the Seq Tube and spin it down briefly.
8. Immediately continue to [2.5 Load flow cell and Olink® Library into reagent cartridge](#).



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

2.5 Load flow cell and Olink® Library into reagent cartridge

During this step, the flow cell and the diluted Olink Library are loaded into the thawed reagent cartridge.

Prepare bench

- 1x Thawed NextSeq™ 1000/2000 P2 Reagent Cartridge (100 cycles), prepared in previous step
- 1x NextSeq™ 1000/2000 P2 Flow Cell, prepared in previous step
- Seq Tube (with ready to load diluted Olink Library), prepared in previous step
- Manual pipette (100 µL)
- Pipette tip (1 mL)

2.5.1 Prepare the cartridge

1. Remove the cartridge from the silver foil bag.
2. Invert the cartridge ten times to thoroughly mix the thawed reagents within.



NOTE: It is normal to hear internal components jingling.

2.5.2 Load the flow cell into the cartridge

1. When ready to load the flow cell into the cartridge, remove the flow cell from the package. Hold the flow cell by the grey tab, with the label on the tab facing up. Use new powder free gloves to avoid contaminating the glass surface of the flow cell.
2. Insert the flow cell into the flow cell slot at the front of the cartridge. An audible click indicates that the flow cell is correctly placed.
3. Remove the grey tab by pulling it out.

2.5.3 Load the Olink® Library into the cartridge

1. Pierce the Library reservoir with a clean 1 mL pipette tip.
2. Load 20 µL of the Olink Library from the Seq Tube into the bottom of the library reservoir.

2.6 Perform Olink® sequencing run

During this step, the Buffer cartridge with the loaded flow cell and Olink Library is loaded into the NextSeq™ 2000, and the sequencing run is started using the Olink recipe.

Prepare bench

- 1x NextSeq™ 1000/2000 P2 Reagent Cartridge (100 cycles) loaded with the NextSeq™ 1000/2000 P2 Flow Cell and the diluted Olink Library, prepared in previous step.

2.6.1 Configure the Run Mode

1. From the control software menu, select *Settings*.
2. Under *BaseSpace Sequence Hub Services & Proactive Support*, select *Local Run Setup*.
3. Select *Proactive Support Only* as additional settings. This function requires an internet connection.
4. Select the *Hosting Location* for your data. Hosting Location should be in or close to your region.
5. Set the *Output Folder* location for the current run raw data. Select *Choose* to navigate and select the output folder.
6. Select the *Denature and Dilute On Board* checkbox to automatically denature and dilute the Library onboard the instrument.
7. Select the *Purge Reagent Cartridge* checkbox to automatically purge unused reagents to the spent reagents compartment of the cartridge.
8. Select the *Autocheck for software updates* checkbox to automatically check for software updates (optional). This function requires an internet connection.
9. Select *Save*.

2.6.2 Set up run parameters



NOTE: This instruction applies to version 1.4 of the NextSeq™ 1000/2000 control software. Some of the steps described below may be different when using version v1.2

1. From the control software menu, select *Start*.
2. Select *Manually Set Up New Run* and press *Setup*.
3. In the *Run Setup* page, set up the run parameters as follows:
 - In the *Run Name* field, enter a unique experiment ID.
 - In the *Read Type* drop-down list, 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



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



NOTE: Ignore the warning messages when entering the number of cycles.

- In the *Custom Primer Wells* drop-down list, select *No*.
- In the *Custom Recipe* (optional) field, select *Choose* to navigate and select the custom recipe XML file *Olink_NSQ2K_P2_V1*. Select *Open*.
- Do not import a Sample Sheet.
- Make sure that the *Output Folder* location is correct. Otherwise, select *Choose* to navigate and select the desired output folder location.

- In the *Denature and Dilute Onboard* field, select *Enabled* from the drop-down list.
- Select *Prep*.


2.6.3 Load the loaded cartridge

1. Select *Load*. The instrument visor opens and the tray is ejected.
2. Place the loaded cartridge onto the tray with the label facing up and the flow cell inside the instrument.
3. Select *Close*.
4. Once the cartridge is properly loaded, verify the run parameters and select *Sequence*. The instrument performs pre-run checks for the instrument and the fluidics.

 **NOTE:** During the fluidics check, it is expected to hear several popping sounds.

5. Make sure that the run starts after the automatic pre-run checks have been completed (~15 minutes). The sequencing run time is approximately 10h30 min.

 **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™ 2000 during the sequencing run. The instrument is sensitive to vibrations.

6. Clean the work area.


2.6.4 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.


2.6.5 Eject and discard the cartridge after the run

 **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 1000 and 2000 System Guide (document #1000000109376).

1. When the run is complete, select *Eject Cartridge*.

 **NOTE:** The used cartridge including flow cell can be left in place until the next run, but no more than 3 days.

2. Remove the cartridge from the tray.
3. Dispose of the reagents in accordance with applicable standards.
4. Select *Close Door*. The tray is reloaded.
5. Select *Home* to return to the Home screen.

 **NOTE:** Since the cartridge contains all the mechanisms to run the system, as well as a reservoir to collect used reagents, there is no need for an instrument wash after the run.

3. Revision history

Version	Date	Description
1.2	2024-07-10	Removed Olink® Explore 1536 Reagent Kit and Olink® Explore Expansion Reagent kit. Document note removed.
1.1	2022-11-01	1.2 Replaced Olink® Mydata with Olink® NPX Explore. 2.2 Edited.
1.0	2021-12-01	New

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