



Olink[®] Explore HT Sequencing using NovaSeq[™] 6000 S4

User Manual

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Part 1: Overview

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 manual provides the instructions needed to sequence Olink® Explore HT libraries on Illumina® NovaSeq™ 6000 S4.

The instructions described in this manual 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 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 Olink® NPX Explore HT User Manual.

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1.2.1 Definition of alert levels


The following alert levels are used in the Olink Explore 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

- Olink® Explore HT User Manual
- Olink® NPX HT Explore User Manual

3. Technical support

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

Part 2: Laboratory instructions

4. Introduction

This chapter provides instructions on how to sequence Olink Libraries prepared using the Olink Explore HT Reagent Kit on NovaSeq™ 6000, using the NovaSeq™ 6000 S4 Reagent Kit v1.5 (35 cycles). The protocol used for sequencing is an adaptation of the Illumina® NovaSeq™ Standard workflow for Illumina® NovaSeq™ 6000.

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


5. Plan the sequencing run

One Olink® Library can be sequenced per NovaSeq™ 6000 S4 flow cell using the Standard workflow, and two Olink® Libraries can be sequenced per run.

An Olink® Library is prepared by pooling at equal volume ratios (1:1) the eight purified libraries that correspond to the 8 different blocks. Refer to the Olink Explore HT User Manual.

6. Install Olink custom recipe

Unzip the custom recipe folder and save the Olink custom recipe XML file (Olink_NovaSeq6K_STND_S4) in an appropriate folder (preferably not in the standard recipe folder).

 **NOTE:** *The Olink custom recipe will only work with the NovaSeq™ 6000 S4 Reagent Kit v1.5 (35 cycles) and the NovaSeq Control Software (NVCS) v1.7 and v1.8. If another NovaSeq Control Software is installed on the instrument, please contact support at support@olink.com.*


7. Prepare sequencing reagents

Prepare bench


- NovaSeq™ 6000 S4 Reagent Kit v1.5 (35 cycles)

Instructions

1. Place the frozen S4 SBS and S4 Cluster (CPE) cartridges half-submerged in room tempered water and let them thaw for 4 hours. Make sure that all reagent reservoirs of the cartridges are completely thawed.

 **NOTE:** *For convenience, thaw the cartridges the day before and store them over night at 4 °C (for up to 24 hours). Thawed reagent cartridges can be re-frozen once.*

2. Dry the cartridge bases thoroughly with a paper towel, and dry the foil seals with a lint-free tissue if needed. Store the cartridges at room temperature if they will be used within 4 hours.

 **NOTE:** *If the reagents will not be used within 4 hours, they can be stored in +4 °C for up to 24 hours.*

8. 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.

8.1 Prepare NaOH dilution

The NaOH dilution is later 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

1. Prepare a 0.2 N NaOH dilution in the 0.2 N NaOH Tube according to:

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

2. Vortex the 0.2 N NaOH Tube thoroughly and spin down. Use within 12 hours.

8.2 Prepare Tris-HCl dilution

The Tris-HCl dilution is later used to neutralize the denatured Libraries.

Prepare bench

- 1 M Tris-HCl pH 8.0 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 400 mM Tris-HCl dilution in the Tris-HCl Tube according to:

Reagent	Volume (µL)
MilliQ water	60
1 M Tris-HCl pH 8.0 stock (Trizma® hydrochloride solution)	40

2. Vortex the Tris-HCl Tube thoroughly and spin down.

8.3 Dilute Olink® Libraries

During this step, the purified and quality controlled Libraries are diluted 1:150.

Prepare bench

- PL Tube, prepared according to the Olink Explore HT User Manual.
- MilliQ 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 tube if frozen.
- Mark the new microcentrifuge tube as "Dil"

Instructions

1. Add 745 µL of MilliQ water into the Dil Tube.
2. Vortex the PL Tube and spin down briefly.
3. Transfer 5 µL from the PL Tube to the Dil Tube.
4. Vortex the Dil Tube and spin down briefly.



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

8.4 Denature Olink® Libraries

During this step, the diluted Libraries are denatured.

Prepare bench

- Dil Tube, prepared in previous step
- 0.2 N NaOH Tube, freshly prepared in previous step
- Tris-HCl Tube, prepared in previous step
- 1x Microcentrifuge tubes (1.5 mL)
- Manual pipettes (0.5–10 µL, 10–100 µL)
- Filter pipette tips

Before you start

- Mark the new microcentrifuge tube as "Den".

Instructions

1. Transfer 310 µL from the Dil Tube to the Den Tube, then add 77 µL of 0.2 N NaOH to the Den Tube.
2. Vortex the Den Tube and spin down briefly.
3. Discard the Dil Tube.
4. Incubate the Den Tube for 8 minutes at room temperature to denature the Library.
5. Transfer 78 µL from the Tris-HCl tube into the Den Tube to neutralize the reaction.
6. Vortex the Den Tube and spin down briefly. Store at +4 °C until ready to continue.

9. Load reagents into NovaSeq™ 6000

During this step, the flow cell, SBS, Cluster and Buffer cartridges are loaded into the NovaSeq™ 6000. The denatured library is loaded into the Library Tube and inserted into the S4 Cluster (CPE) cartridge.

Prepare bench

- 1x S4 Flow cell
- NovaSeq™ 6000 S4 Reagent kit(s) v1.5 (35 cycles), prepared in previous step, including Library Tube
- Lint-free isopropyl wipes
- Low-lint lab tissue

Before you start

- Let the refrigerated flow cell foil package(s) reach room temperature for 10-15 minutes. Do not remove the seals.
- Make sure that the S4 SBS and S4 Cluster (CPE) cartridges are completely thawed and thoroughly dried.
- Remove any items from the NovaSeq™ 6000 instrument.

9.1 Load flow cells into NovaSeq™ 6000

1. On the NovaSeq™ 6000 Home screen, select Sequence.
2. Select the applicable protocol: Single flow cell run on side A (A) or on side B (B).
3. On the Load run setup screen, select OK to open the flow cell door.
4. Select Close Flow Cell Door. The sensors and RFIDs are checked, and the flow cell RFIDs are displayed on the screen



NOTE: *If the flow cell RFID does not appear on the screen, wipe the flow cell barcode and the flow cell compartment with a lint-free isopropyl wipe and then reload the flow cell.*

9.2 Load SBS and Cluster cartridges into NovaSeq™ 6000

1. Invert the S4 SBS and S4 Cluster (CPE) cartridges ten times to mix the reagents, then gently tap the bottom of the cartridges against the bench to reduce air bubbles.
2. Open the liquid compartment and reagent chiller doors of the NovaSeq™ 6000.
3. Remove the used SBS and Cluster cartridges.
4. Uncap the Library Tube provided with the NovaSeq™ 6000 S4 Reagent Kit.
5. Load all the denatured Olink Library in the Den tube (~465 µL) into the bottom of the Library Tube.
6. Gently tap the Library Tube against the bench to reduce air bubbles.
7. Insert the Library Tube into the Library Tube position (#8) of the new S4 Cluster (CPE) cartridge.



8. Load the prepared cartridges into the reagent chiller drawer with the “Insert” labels facing towards the back of the instrument:
 - a. Place the S4 SBS cartridge (gray label) to the left.
 - b. Place the S4 Cluster (CPE) cartridge (orange label) containing the library tube to the right.
9. Close the liquid compartment and reagent chiller doors.

9.3 Load buffer cartridge into NovaSeq™ 6000

1. Open the buffer drawer.
2. Remove the used buffer cartridge from the buffer drawer. The used buffer cartridge has pierced foil seals.
3. Place a new buffer cartridge into the buffer drawer with the “Illumina” label facing the front of the drawer. Align the cartridge with the raised guides on the drawer floor and sides. Make sure that the buffer cartridge is evenly seated and that the drawer can be closed.
4. Empty the reagent bottles:
 - a. Seal the small reagent bottle to prevent spilling. Remove the bottle from the alcove and discard the content in accordance with applicable standards.

⚠ WARNING: The small used reagent bottle contains Formamide. When removing the bottle from the alcove, seal the bottle with the cap attached to the bottle to prevent spilling.

- b. Return the small bottle (uncapped) to the alcove.
- c. Seal the large reagent bottle with the attached cap to prevent spilling. Remove the bottle from the alcove and discard the content in accordance with applicable standards.
- d. Return the large bottle (uncapped) to the alcove.

⚠ WARNING: Failure to empty the used reagent bottles can result in a terminated run and overflow, which may damage the instrument and pose a safety risk.

5. Check the checkbox acknowledging that both of the reagent bottles are empty.

10. Perform Olink® sequencing run

During this step, the sequencing run is started using the Olink custom recipe.

10.1 Set up run parameters and start sequencing run

1. Select Run Setup.
2. Ensure that the NovaSeq™ Standard workflow is selected (make sure that the library tube is loaded and filled with the diluted and denatured library).
3. In the Run Name field, enter a unique experiment ID.
4. Do not edit the Cycle Number Fields, leave as default values.
5. Expand Advanced Options to apply settings for the current run. Under Output Folder, select Browse to change the output folder for the current run raw data.
6. Select Custom Recipe, then Browse to use the Custom Recipe XML file for the run: Olink_NovaSeq6K_STND_S4.
7. Select Review. The software confirms that the specified parameters are appropriate for the recipe. The Review screen will show the updated cycle numbers (24, 0, 0, 0) as defined in the recipe.
8. Confirm the run parameters displayed on the Review screen. To edit any parameters, press Back to return to the Run Setup screen.
9. Select Start Run. Make sure that the run starts after the automatic pre-run checks have been completed (~5 minutes). The sequencing run time is approximately 9h30 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 NovaSeq™ 6000 during the sequencing run. The instrument is sensitive to vibrations.

10. Clean the work area. If needed, clean the flow cell dock with a lint-free isopropyl wipe and dry it with a low-lint lab tissue before returning it to storage.
11. When the sequencing is completed, the software initiates an automatic post-run wash that takes about 80 minutes. When the wash is completed, the system is placed in a safe state and the Home button turns active. Leave all consumables in place until the next run.

10.2 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.

11. Revision history

Version	Date	Description
1.1	2023-12-12	6 and 10.1: Olink_NovaSeq6K_STND_S4 renamed. Editorial changes
1.0	2023-08-29	New

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