



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

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The Olink® Explore User manual, doc nr 1153, is obsolete, and has been replaced by the following documents: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® NovaSeq™ 6000. For libraries prepared using any of the Olink® Explore Reagent Kits, refer to [2. Laboratory instruction for Olink® Explore \(96 samples\)](#). For libraries prepared using any of the Olink® Explore Reagent Kits (384 samples), refer to [3. Laboratory instructions for Olink® Explore \(384 samples\)](#).

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 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 instruction for Olink® Explore (96 samples)

This chapter provides instructions on how to sequence Olink Libraries prepared using any of the Olink Explore Reagent Kits on NovaSeq™ 6000, using the NovaSeq™ 6000 SP Reagent Kit v1.5 (100 cycles). The protocol used for sequencing is an adaptation of the Illumina® NovaSeq™ Xp workflow for Illumina® NovaSeq™ 6000.

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

2.1 Plan the sequencing run

Two Olink® Libraries can be sequenced per NovaSeq™ 6000 SP flow cell, and up to four Olink Libraries can be sequenced per run. The number of SP flow cells required to sequence the different Olink Explore Reagent Kits is described in [Table 1](#).

If running Olink Explore 3072, perform the instruction described in [2. Laboratory instruction for Olink® Explore \(96 samples\)](#) twice, once for each sequencing run.

Table 1. Sequencing run planning using SP flow cell(s).

Olink Explore Reagent Kit	Number of Olink® Libraries	Number of SP flow cell(s)
Olink Explore 384 Reagent Kit	1	1
Olink Explore 4 x 384 Reagent Kit	4	2
Olink Explore 1536 Reagent Kit	4	2
Olink Explore Expansion Reagent Kit	4	2
Olink Explore 3072 Reagent Kit	8	4

2.2 Prepare Olink® custom recipe

During this step, the Olink® custom recipe is installed on the NovaSeq™ 6000. This step only needs to be performed once, before an Olink sequencing run is performed for the first time.

Unzip the Custom recipe folder and save the Olink custom recipe xml-file in an appropriate folder (preferably not in the standard recipe folder).



NOTE: The Olink custom recipe will only work with the NovaSeq™ 6000 SP Reagent Kit v1.5 (100 cycles) and the NovaSeq Control Software (NVCS) v1.7 and v1.7.5. If another NovaSeq Control Software is installed on the instrument, please contact support at support@olink.com.

2.3 Prepare sequencing reagents

During this step, sequencing reagents are thawed.



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 NovSeq 6000 System Guide (document #1000000019358).

Prepare bench

- NovaSeq™ 6000 SP Reagent Kit v1.5 (100 cycles):
 - For Olink Explore 384 single panel: 1x
 - For Olink Explore 4 x 384, Expansion, 1536 or 3072: 2x

Instructions

1. Place the frozen SP SBS and SP 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: Thaw the cartridges the day before and store them over night at 4 °C (for up to 24 hours).

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. Thawed reagent cartridges can be re-frozen once.

2.4 Prepare Olink® Library for sequencing

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

2.4.1 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 [Table 2](#).
2. 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 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 [Table 3](#).
2. Vortex the Tris-HCl Tube thoroughly and spin down.

Table 3. 400 mM Tris-HCl dilution

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

2.4.3 Dilute Olink® Libraries

During this step, the purified and quality controlled Libraries are diluted 1:100.

Prepare bench

- Lib Tube(s), prepared according to the applicable Olink Explore User Manual
- MilliQ water
- Microcentrifuge tube(s) (1.5 mL)
 - For Olink® Explore 384 single panel: 1x
 - For Olink® Explore 4 x 384, Expansion, 1536 or 3072: 4x
- Manual pipettes (0.5–10 µL, 100–1000 µL)
- Filter pipette tips

Before you start


- Thaw the Lib Tube(s) if frozen.
- Mark the new microcentrifuge tube(s):
 - For Olink® Explore 384 single panel: “Dil 1”
 - For Olink® Explore 4 x 384, Expansion, 1536 or 3072: “Dil 1”–“Dil 4”

Instructions

1. Add 495 µL of MilliQ water to each Dil Tube.
2. Vortex the Lib Tube(s) and spin down briefly.
3. Transfer 5 µL from each Lib Tube to the corresponding Dil Tube according to [Table 4](#).
4. Vortex the Dil Tube(s) and spin down briefly.

Table 4. Transfer Libraries to Dil Tubes

Volume (μL)	From tube	To tube	Comment
5	Lib 1	Dil 1	Only applicable if running Olink® Explore 4 x 384, Expansion, 1536 or 3072
5	Lib 2	Dil 2	
5	Lib 3	Dil 3	
5	Lib 4	Dil 4	

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

2.4.4 Denature Olink® Libraries

During this step, the diluted Libraries are denatured.

Prepare bench

- Dil Tube(s), prepared in previous step
- 0.2 N NaOH Tube, freshly prepared in previous step
- Tris-HCl Tube, prepared in previous step
- Microcentrifuge tube(s) (1.5 mL):
 - For Olink Explore 384 single panel: 1x
 - For Olink Explore 4 x 384, Expansion, 1536 or 3072: 4x
- Manual pipettes (0.5–10 μL, 10–100 μL)
- Filter pipette tips

Before you start

- Mark the new microcentrifuge tube(s):
 - For Olink Explore 384 single panel: “Den 1”.
 - For Olink Explore 4 x 384, Expansion, 1536 or 3072: “Den 1”–“Den 4”.

Instructions

1. Transfer 18 μL from each Dil Tube to the corresponding Den Tube, then add 4 μL of 0.2 N NaOH to each Den Tube according to [Table 5](#).

Table 5. Denature Libraries


Volume (μL)	From tube	To tube	Comment
18	Dil 1	Den 1	
4	0.2 N NaOH		
18	Dil 2	Den 2	Only applicable if running Olink Explore 4 x 384, Expansion, 1536 or 3072
4	0.2 N NaOH		
18	Dil 3	Den 3	
4	0.2 N NaOH		
18	Dil 4	Den 4	
4	0.2 N NaOH		

2. Vortex the Den Tube(s) and spin down briefly.
3. Discard the Dil Tube(s).
4. Incubate the Den Tube(s) for 8 minutes at room temperature to denature the Libraries.
5. Transfer 5 μL from the Tris-HCl Tube to each Den Tube to neutralize the reaction. Change pipette tip between each tube.
6. Vortex the Den Tube(s) and spin down briefly. Store at +4 °C until ready to proceed to [2.6 Load Libraries onto flow cells](#).

2.5 Prepare flow cells and flow cell docks

During this step, the flow cell(s) are placed in the flow cell dock(s) and equipped with manifold(s). Perform the instruction below once per flow cell.

 **NOTE:** Keep the flow cells level at all times until loaded into the instrument.

 **NOTE:** If there is only one flow cell dock available, perform both [2.5 Prepare flow cells and flow cell docks](#) and [2.6 Load Libraries onto flow cells](#) for the first flow cell, then repeat both instructions for the second flow cell.

Prepare bench

- SP flow cell(s):
 - For Olink Explore 384 single panel: 1x
 - For Olink Explore 4 x 384, Expansion, 1536 or 3072: 2x
- NovaSeq™ Xp 2-Lane Kit(s):
 - For Olink Explore 384 single panel: 1x
 - For Olink Explore 4 x 384, Expansion, 1536 or 3072: 2x
- Lint-free isopropyl alcohol wipe.
- 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.

Instructions

1. Place the NovaSeq™ Xp flow cell dock on a flat surface ([Figure 1](#)).

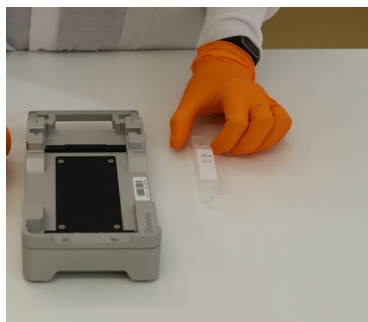



Figure 1. NovaSeq™ XP flow cell dock

2. Put on new powder free gloves (to avoid contaminating the glass surface of the flow cell).
3. Peel open the foil of the flow cell package from the corner tab.
4. Remove the clear plastic retainer covering the flow cell.

 **NOTE:** Use the flow cell within 12 hours after removal from the package.

5. Grasp the flow cell by the sides and remove it from the package. Do not touch the glass or the underside gaskets.
6. 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.
7. Perform a functional test of the flow cell using the NovaSeq™ instrument:
 - a. In the NovaSeq™ Control Software, click *Sequence*, then *Open flow cell door*.
 - b. Place the new, unloaded flow cell in the NovaSeq™ and close the flow cell door. Make sure that a green check mark appears in the software, indicating that the system can create a vacuum and that the software can read the barcode of the flow cell.

 **NOTE:** If error messages appear, try reloading the flow cell. If it still does not work, wipe the flow cell compartment gently with a lint-free isopropyl wipe and reload the flow cell.

c. Open the flow cell door and remove the flow cell.

8. Invert the flow cell so that the top surface faces downward. Slip the outlet end of the flow cell under the bracket at the top of the NovaSeq™ Xp flow cell dock. Place the flow cell on the dock (*Figure 2*).

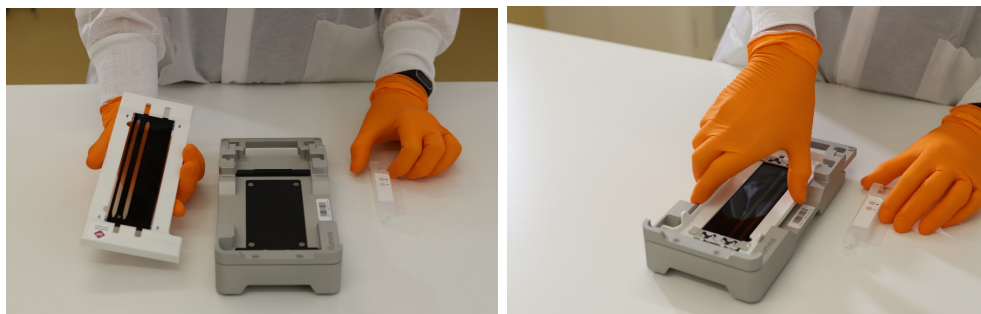


Figure 2. Placing flow cell on the dock

9. Mount the manifold over the inlet end of the flow cell, with the wells facing up (*Figure 3*). Make sure that the manifold arms fit securely into the dock cutouts.

 **IMPORTANT:** Do not reuse manifolds.

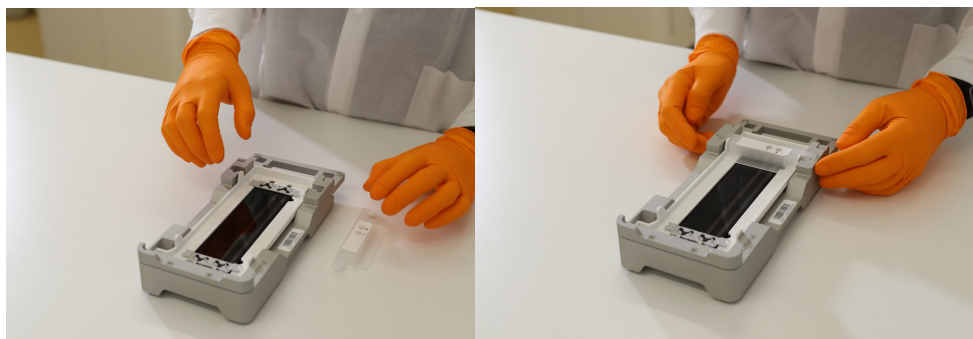


Figure 3. Mounting manifold over inlet

10. Close the clamp to secure the flow cell and manifold (*Figure 4*). Seal the gaskets.



Figure 4. Closing the clamp

11. Repeat the instruction as necessary to prepare a second flow cell.

2.6 Load Libraries onto flow cells

During this step, an ExAmp Master Mix is prepared and added to the denatured Libraries, which are then loaded onto the flow cell(s). Prepare one ExAmp Master Mix eppendorf tube per flowcell.

Prepare bench

- Den Tube(s), prepared in previous step
- ExAmp reagents JPX1, JPX2, DPX3
- Flow cell(s) with manifold(s), placed in flow cell dock(s)
- Microcentrifuge tube(s) (1.5 mL):
 - For Olink Explore 384 single panel: 2x
 - For Olink Explore 4 x 384, Expansion, 1536 or 3072: 6x
- Manual pipette (20–200 µL)
- Filter pipette tips

Before you start

- Mark the new microcentrifuge tube(s):
 - For Olink Explore 384 single panel, mark two tubes “ExAmp Mix 1” and “Seq 1”
 - For Olink Explore 4 x 384, Expansion, 1536 or 3072, mark six tubes “ExAmp Mix 1” - “ExAmp Mix 2” and “Seq 1”–“Seq 4”
- Make sure that the ExAmp reagents are completely thawed

Instructions

1. Prepare the ExAmp Master Mix(es) in the ExAmp Mix Tube(s) according to [Table 6](#).
 - a. Vortex each reagent and spin briefly before adding it to the mix
 - b. Pipette carefully from the surface to avoid formation of air bubbles and liquid sticking to the outside of the tip
 - c. Make sure that the whole volume is dispensed from the tip
 - d. Pipette up and down a few times after dispensing to wash out the tip



NOTE: The reagent's viscosity increases with the reagent number.

Table 6. ExAmp Master Mix

Addition order	ExAmp Reagents	Volume (µL)
1	JPX1	126
2	JPX2	18
3	DPX3	66
Total		210

2. Vortex the ExAmp Mix Tube for 20–30 seconds, and spin briefly. For best sequencing performance, immediately proceed to the next step.




NOTE: If necessary, store the ExAmp Master Mix(es) for up to 1 hour on ice. If stored at room temperature, it must be used within 30 minutes.

3. Transfer 63 µL of ExAmp Master Mix into each Seq Tube, then transfer 27 µL from each Den Tube to the corresponding Seq Tube according to [Table 7](#).

Table 7. Transfer denatured Libraries to Seq Tubes

Volume (μL)	From tube	To tube	Comment
63	ExAmp Mix 1	Seq 1	
27	Den 1		
63	ExAmp Mix 1	Seq 2	Only applicable if running Olink Explore 4 x 384, Expansion, 1536 or 3072
27	Den 2		
63	ExAmp Mix 2	Seq 3	
27	Den 3		
63	ExAmp Mix 2	Seq 4	
27	Den 4		

- Vortex the Seq Tube(s) and spin briefly. Immediately continue to the next step.
- Transfer 80 μL from the Seq 1 Tube, and from the Seq 2 Tube if applicable, to the appropriate manifold well(s) of flow cell 1 according to [Table 8](#) (flow cell 2 will be filled later). Use forward pipetting, but do not push the operating button to the second stop. Change pipette tip between each Seq Tube.

 **NOTE:** Make sure to add the library to the intended well. As the flow cell is inverted, the well numbering is reversed (going from right to left).


 **NOTE:** Pipette carefully to avoid creating air bubbles in the manifold. Avoid contact with the filter at the bottom of the well.

Table 8. Load Libraries onto flow cell(s)

Flow cell	Volume (μL)	From tube	To manifold well
1	80	Seq 2	2 (left)
	80	Seq 1	1 (right)
2	80	Seq 4	2 (left)
	80	Seq 3	1 (right)

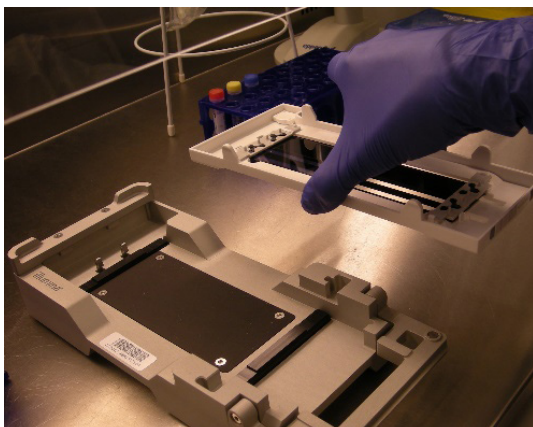


- Wait approximately 2 minutes for the loaded Libraries to reach the opposite end of the flow cell lanes. It is normal that a small air bubble forms at the outlet end of the lane, or that a small volume of loaded library remains in the manifold wells.

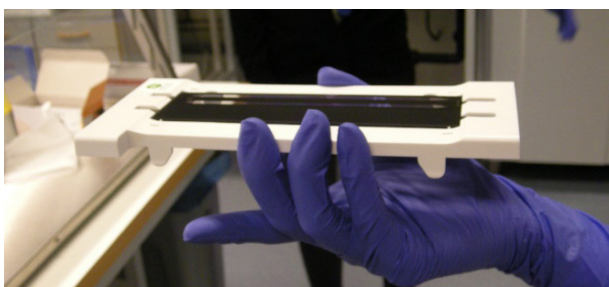
 **NOTE:** Make sure to keep the flow cell level. Tilting may cause leakage.

- Remove the flow cell(s) from the flow cell dock(s):
 - Open the clamp that secures the flow cell and manifold.
 - Carefully remove and discard the manifold.

- c. Grasp the sides of the flow cell and remove it from the dock.



8. Invert the flow cell around the long axis so that the top surface faces up.



9. Place the loaded flow cell(s) in the flow cell compartment of the NovaSeq™ 6000 as described in [2.7.1 Load flow cells into NovaSeq™ 6000](#).
10. If applicable, repeat steps 6-10 to load Seq 3 and Seq 4 Tubes onto flow cell 2.



TIME SENSITIVE STEP: Start the sequencing run within 30 minutes after loading the first flow cell.

2.7 Load reagents into NovaSeq™ 6000

During this step, the flow cell(s), SBS, Cluster and Buffer cartridges are loaded into the NovaSeq™ 6000.

Prepare bench


- Flow cell(s), prepared in previous step
- NovaSeq™ 6000 SP Reagent Kit(s) v1.5 (100 cycles), prepared in previous step
- Lint-free isopropyl wipes
- Low-lint lab tissue

Before you start

- Make sure that the SP SBS and SP Cluster (CPE) cartridges are completely thawed and thoroughly dried.
- Remove any items from the NovaSeq™ 6000 instrument.

2.7.1 Load flow cells into NovaSeq™ 6000

1. On the NovaSeq™ 6000 *Home* screen, select *Sequence*.
2. Select the applicable protocol:
 - For Olink Explore 384 single panel: *Single flow cell run on side A (A) or on side B (B)*.
 - For Olink Explore 4 x 384, Expansion, 1536 or 3072: *Dual flow cell run (A+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.

 **NOTE:** It is not necessary to close the flow cell door until the second flow cell has been placed in the instrument.

2.7.2 Load SBS and Cluster cartridges into NovaSeq™ 6000

1. Invert the SP SBS and SP 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 tubes provided with the NovaSeq™ 6000 SP Reagent Kits. Insert one library tube into the Library Tube position (#8) of each new SP Cluster (CPE) cartridge (for an Olink run with dual flow cells, one of each reagent cartridge is required on each side of the instrument).
5. Load the prepared cartridges into the reagent chiller drawer with the “Insert” labels facing towards the back of the instrument:
 - a. Place the SP SBS cartridge (gray label) to the left.
 - b. Place the SP Cluster (CPE) cartridge (orange label) containing the library tube to the right.
6. Close the liquid compartment and reagent chiller doors.

2.7.3 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.

2.8 Perform Olink® sequencing run

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

2.8.1 Set up run parameters and start sequencing run

1. Select *Run Setup*.
2. Make sure that the *NovaSeq™ Xp* workflow is selected.
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_SP_V1*.
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, 1, 1, 1) 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 7h15 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.

2.8.2 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 Olink 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. Laboratory instructions for Olink® Explore (384 samples)

This chapter provides instructions on how to sequence Olink Libraries prepared using any of the Olink Explore Reagent Kits (384 samples) 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™ Xp workflow for Illumina® NovaSeq™ 6000.

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

3.1 Plan the sequencing run

Four 384-sample Olink Libraries can be sequenced per NovaSeq™ 6000 S4 flow cell, and up to eight 384-sample Olink Libraries can be sequenced per run. The number of flow cells required to sequence the different Olink Explore Reagent Kits is described in [Table 9](#).

Table 9. Sequencing run planning using S4 flow cell(s).

Olink Explore Reagent Kit	Number of 384-sample Olink Libraries	Number of S4 flow cell(s)
Olink Explore 1536 Reagent Kit (384 samples)	4	1
Olink Explore Expansion Reagent Kit (384 samples)	4	1
Olink Explore 3072 Reagent Kit (384 samples)	8	2

3.2 Install Olink® custom recipe

Unzip the custom recipe folder and save the Olink custom recipe xml-file 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 v1.7.5. If another NovaSeq Control Software is installed on the instrument, please contact support at support@olink.com.

3.3 Prepare sequencing reagents

During this step, sequencing reagents are thawed.



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 NovaSeq 6000 System Guide (document #1000000019358).


Prepare bench

- NovaSeq™ 6000 S4 Reagent Kit v1.5 (35 cycles):
 - For Olink Explore Expansion or 1536 (384 samples): 1x
 - For Olink Explore 3072 (384 samples): 2x

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.

3.4 Prepare Olink® Libraries for sequencing

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

3.4.1 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 [Table 10](#).
2. Vortex the 0.2 N NaOH Tube thoroughly and spin down. Use within 12 hours.

Table 10. 0.2 N NaOH dilution

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

3.4.2 Prepare Tris-HCl dilution

The Tris-HCl dilution is later used to neutralize the denaturated 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 [Table 11](#).
2. Vortex the Tris-HCl Tube thoroughly and spin down.

Table 11. 400 mM Tris-HCl dilution

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

3.4.3 Dilute Olink® Libraries

During this step, the purified and quality controlled Libraries are diluted 1:100.

Prepare bench

- PL Tubes, prepared according to the applicable Olink Explore User Manual
- MilliQ water
- Microcentrifuge tubes (1.5 mL)
 - For Olink Explore Expansion or 1536 (384 samples): 4x
 - For Olink Explore 3072 (384 samples): 8x
- Manual pipettes (0.5–10 μL, 100–1000 μL)
- Filter pipette tips

Before you start

- Thaw the PL Tubes if frozen.
- Mark the new microcentrifuge tubes:
 - For Olink Explore Expansion or 1536 (384 samples): “Dil 1”–“Dil 4”
 - For Olink Explore 3072 (384 samples): “Dil 1”–“Dil 8”

Instructions

1. Add 495 μL of MilliQ water into each Dil Tube.
2. Vortex the PL Tubes and spin down briefly.
3. Transfer 5 μL from each PL Tube to the corresponding Dil Tube according to [Table 12](#).

Table 12. Transfer Libraries to Dil Tubes

Volume (μL)	From tube	To tube	Comment
5	PL 1	Dil 1	
5	PL 2	Dil 2	
5	PL 3	Dil 3	
5	PL 4	Dil 4	
5	PL 5	Dil 5	Only applicable if running Olink Explore 3072 (384 samples)
5	PL 6	Dil 6	
5	PL 7	Dil 7	
5	PL 8	Dil 8	

- Vortex the Dil Tubes and spin down briefly.

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

3.4.4 Denature Olink® Libraries

During this step, the diluted Libraries are denatured.

Prepare bench

- Dil Tubes, prepared in previous step
- 0.2 N NaOH Tube, freshly prepared in previous step
- Tris-HCl Tube, prepared in previous step
- Microcentrifuge tubes (1.5 mL):
 - For Olink Explore Expansion or 1536 (384 samples): 4x
 - For Olink Explore 3072 (384 samples): 8x
- Manual pipettes (0.5–10 µL, 10–100 µL)
- Filter pipette tips

Before you start

- Mark the new microcentrifuge tubes:
 - For Olink Explore Expansion or 1536 (384 samples): “Den 1”– “Den 4”
 - For Olink Explore 3072 (384 samples): “Den 1”–“Den 8”

Instructions

- Transfer 30 µL from each Dil Tube to the corresponding Den Tube, then add 7 µL of 0.2 N NaOH to each Den Tube, according to [Table 13](#).

Table 13. Denature Libraries

Volume (µL)	From tube	To tube	Comment
30	Dil 1	Den 1	
7	0.2 N NaOH		
30	Dil 2	Den 2	
7	0.2 N NaOH		
30	Dil 3	Den 3	
7	0.2 N NaOH		
30	Dil 4	Den 4	
7	0.2 N NaOH		
30	Dil 5	Den 5	Only applicable if running Olink Explore 3072 (384 samples)
7	0.2 N NaOH		
30	Dil 6	Den 6	
7	0.2 N NaOH		
30	Dil 7	Den 7	
7	0.2 N NaOH		
30	Dil 8	Den 8	
7	0.2 N NaOH		


- Vortex the Den Tubes and spin down briefly.
- Discard the Dil Tubes.

4. Incubate the Den Tubes for 8 minutes at room temperature to denature the Libraries.
5. Transfer 8 μ L from the Tris-HCl tube into each Den Tube to neutralize the reaction. Change pipette tip between each tube.
6. Vortex the Den Tubes and spin down briefly. Store at +4 °C until ready to continue to [3.6 Load Libraries onto flow cells](#).

3.5 Prepare flow cells and flow cell docks

During this step, the flow cell(s) are placed in the flow cell dock(s) and equipped with manifold(s). Perform the instruction below once per flow cell.

 **NOTE:** Keep the flow cells level at all times until loaded into the instrument.

 **NOTE:** If there is only one flow cell dock available, perform both [3.5 Prepare flow cells and flow cell docks](#) and [3.6 Load Libraries onto flow cells](#) for the first flow cell, then repeat both instructions for the second flow cell.

Prepare bench

- S4 flow cell(s):
 - For Olink Expansion or 1536 (384 samples): 1x
 - For Olink Explore 3072 (384 samples): 2x
- NovaSeq™ 6000 XP 4-lane kit(s):
 - For Olink Expansion or 1536 (384 samples): 1x
 - For Olink Explore 3072 (384 samples): 2x
- Lint-free isopropyl alcohol wipe
- Low-lint lab tissue

 **IMPORTANT:** Do not reuse manifolds.

Before you start

- Let the refrigerated flow cell foil package(s) reach room temperature for 10-15 minutes. Do not remove the seals.

Instructions

1. Place the NovaSeq™ Xp flow cell dock on a flat surface ([Figure 5](#)).

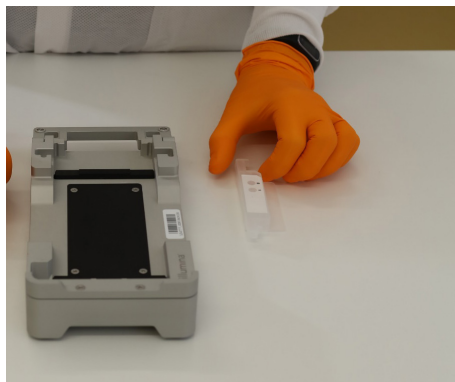



Figure 5. NovaSeq™ XP flow cell dock

2. Put on new powder free gloves (to avoid contaminating the glass surface of the flow cell).
3. Peel open the foil of the flow cell package from the corner tab.
4. Remove the clear plastic retainer covering the flow cell.

 **NOTE:** Use the flow cell within 12 hours after removal from the package.

5. Grasp the flow cell by the sides and remove it from the package. Do not touch the glass or the underside gaskets.
6. 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.
7. Perform a functional test of the flow cell using the NovaSeq™ instrument:
 - a. In the NovaSeq™ Control Software, click *Sequence*, then *Open flow cell door*.
 - b. Place the new, unloaded flow cell in the NovaSeq™ and close the flow cell door. Make sure that a green check mark appears in the software, indicating that the system can create a vacuum and that the software can read the barcode of the flow cell.

 **NOTE:** If error messages appear, try reloading the flow cell. If it still does not work, wipe the flow cell compartment gently with a lint-free isopropyl wipe and reload the flow cell.

- c. Open the flow cell door and remove the flow cell.
8. Invert the flow cell so that the top surface faces downward. Slip the outlet end of the flow cell under the bracket at the top of the NovaSeq™ Xp flow cell dock. Place the flow cell on the dock (*Figure 6*).

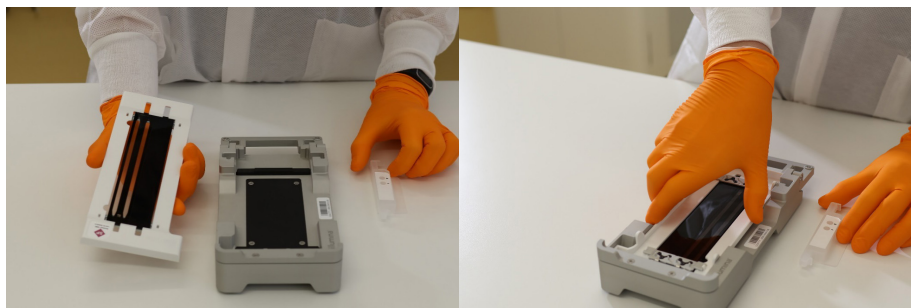


Figure 6. Placing flow cell on the dock

9. Mount the manifold over the inlet end of the flow cell, with the wells facing up (*Figure 7*). Make sure that the manifold arms fit securely into the dock cutouts.

 **IMPORTANT:** Do not reuse manifolds.

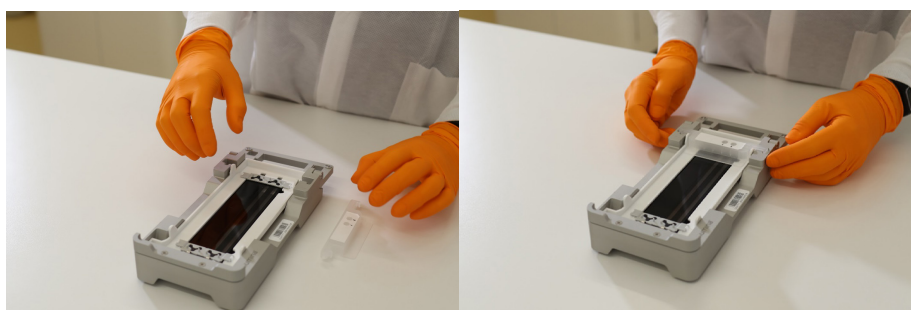


Figure 7. Mounting manifold over inlet

10. Close the clamp to secure the flow cell and manifold (*Figure 8*). Seal the gaskets.

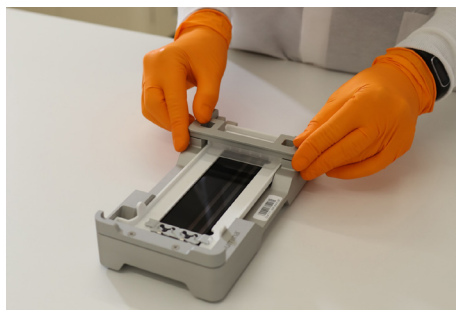


Figure 8. Closing the clamp

11. Repeat the instruction as necessary to prepare a second flow cell.

3.6 Load Libraries onto flow cells

During this step, an ExAmp Master Mix is prepared and added to the denatured Libraries, which are then loaded onto the flow cell(s). Prepare one ExAmp Master Mix eppendorf tube per flowcell.

Prepare bench

- Den Tubes, prepared in previous step
- ExAmp reagents JPX1, JPX2, DPX3
- Flow cell(s) with manifold(s), placed in flow cell dock(s)
- Microcentrifuge tubes (1.5 mL):
 - For Olink Explore 1536 or Expansion (384 samples): 5x
 - For Olink Explore 3072 (384 samples): 10x
- Manual pipette (20–200 µL)
- Filter pipette tips

Before you start

- Mark the new microcentrifuge tube(s):
 - For Olink Explore 1536 or Expansion (384 samples): "ExAmp Mix" and "Seq 1"–"Seq 4"
 - For Olink Explore 3072 (384 samples): "ExAmp Mix" and "Seq"–"Seq 8"
- Make sure that the ExAmp reagents are completely thawed

Instructions

1. Prepare the ExAmp Master Mix in the ExAmp Mix Tube according to [Table 14](#). Prepare one ExAmp Master Mix per flow cell.
 - a. Vortex each reagent and spin briefly before adding it to the mix
 - b. Pipette carefully from the surface to avoid formation of air bubbles and liquid sticking to the outside of the tip
 - c. Make sure that the whole volume is dispensed from the tip
 - d. Pipette up and down a few times after dispensing to wash out the tip



NOTE: The reagent's viscosity increases with the reagent number.

Table 14. ExAmp Master Mix

Addition order	ExAmp Reagents	Volume µL)
1	JPX1	315
2	JPX2	45
3	DPX3	165
	Total	525

- Vortex the ExAmp Mix Tube for 20–30 seconds, and spin briefly. For best sequencing performance, immediately proceed to the next step.



NOTE: If necessary, store the ExAmp Master Mix for up to 1 hour on ice. If stored at room temperature, it must be used within 30 minutes.

- Transfer 105 µL of ExAmp Master Mix into each Seq Tube.
- Transfer 45 µL from each Den Tube to the corresponding Seq Tube according to [Table 15](#).

Table 15. Transfer denatured Libraries to Seq Tubes

Volume (µL)	From tube	To tube	Comment
45	Den 1	Seq 1	
45	Den 2	Seq 2	
45	Den 3	Seq 3	
45	Den 4	Seq 4	
45	Den 5	Seq 5	Only applicable if running Olink Explore 3072 (384 samples)
45	Den 6	Seq 6	
45	Den 7	Seq 7	
45	Den 8	Seq 8	

- Vortex the Seq Tubes and spin briefly. Immediately continue to the next step.
- Transfer 130 µL from each Seq Tube to the corresponding manifold well of the S4 flow cell(s) according to [Table 16](#). Use forward pipetting, but do not push the operating button to the second stop. Change pipette tip between each library.



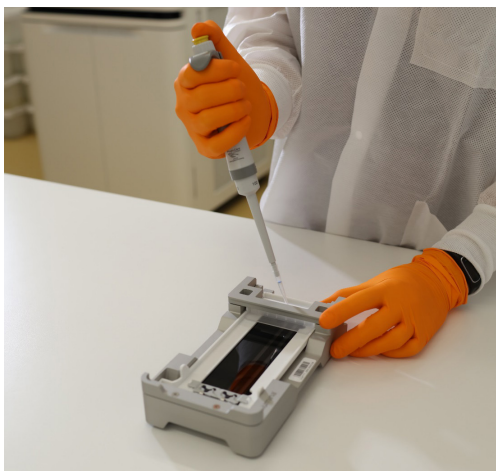
NOTE: Make sure to add the library to the intended well. As the flow cell is inverted, the well numbering is reversed (going from right to left).



NOTE: Pipette carefully to avoid creating air bubbles in the manifold. Avoid contact with the filter at the bottom of the well.

Table 16. Transfer Libraries to flow cell(s)

Flow cell	Volume (µL)	From tube	To manifold well	Comment
1	130	Seq 1	4	
	130	Seq 2	3	
	130	Seq 3	2	
	130	Seq 4	1	
2	130	Seq 5	4	Only applicable if running Olink Explore 3072 (384 samples)
	130	Seq 6	3	
	130	Seq 7	2	
	130	Seq 8	1	

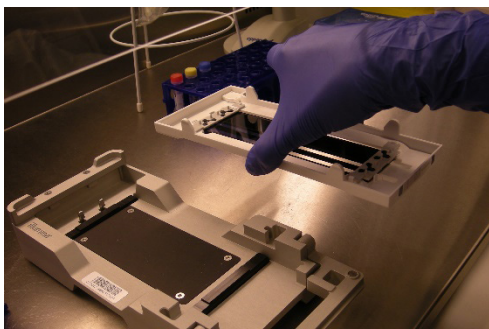


7. Wait approximately 2 minutes for the loaded Libraries to reach the opposite end of the flow cell lanes. It is normal that a small air bubble forms at the outlet end of the lane, or that a small volume of loaded library remains in the manifold wells.

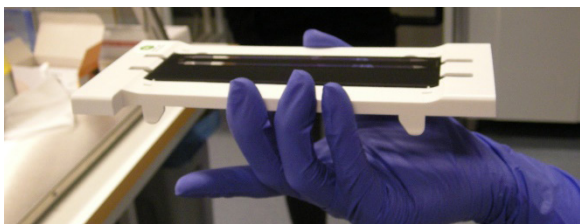


NOTE: Make sure to keep the flow cell level. Tilting may cause leakage.

8. Remove the flow cell(s) from the flow cell dock(s):
 - a. Open the clamp that secures the flow cell and manifold.
 - b. Carefully remove and discard the manifold.
 - c. Grasp the sides of the flow cell and remove it from the dock.



9. Invert the flow cell around the long axis so that the top surface faces up.



10. Place the loaded flow cell(s) in the flow cell compartment of the NovaSeq™ 6000 as described in [3.7.1 Load flow cells into NovaSeq™ 6000](#).



TIME SENSITIVE STEP: Start the sequencing run within 30 minutes after loading the first flow cell.

3.7 Load reagents into NovaSeq™ 6000

During this step, the flow cell(s), SBS, Cluster and Buffer cartridges are loaded into the NovaSeq™ 6000.

Prepare bench

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

Before you start

- 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.

3.7.1 Load flow cells into NovaSeq™ 6000

1. On the NovaSeq™ 6000 *Home* screen, select *Sequence*.
2. Select the applicable protocol.
 - For Olink Explore Expansion or 1536 (384 samples): *Single flow cell run on side A (A) or on side B (B)*.
 - For Olink Explore 3072 (384 samples): *Dual flow cell run (A+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.



NOTE: It is not necessary to close the flow cell door until the second flow cell has been placed in the instrument.

3.7.2 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 tubes provided with the NovaSeq™ 6000 S4 Reagent Kits. Insert one library tube into the Library Tube position (#8) of each new S4 Cluster (CPE) cartridge.
5. 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.
6. Close the liquid compartment and reagent chiller doors.

3.7.3 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.

WARNING:

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.

3.8 Perform Olink® sequencing run

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

3.8.1 Set up run parameters and start sequencing run

1. Select *Run Setup*.
2. Ensure that the *NovaSeq™ Xp* workflow is selected (make sure that an empty library tube is loaded).
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_S4_V1*.
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, 1, 1, 1) 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.

3.8.2 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.

4. Revision history

Version	Date	Description
1.1	2022-11-01	1.2 Replaced Olink® Mydata with Olink® NPX Explore. Editorial changes.
1.0	2021-12-01	New

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Olink Proteomics, Dag Hammarskjölds väg 52B, SE-752 37 Uppsala, Sweden

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