Olink® Explore Overview

User Manual

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Part 1: Olink® Explore overview

1.Introduction

Olink® Explore is a high-multiplex, high-throughput protein biomarker platform intended to measure the relative concentration of proteins in liquid biopsies. The platform uses Olink's PEA™ technology coupled to an innovative new readout methodology based on Next Generation Sequencing (NGS). The protocol is semi-automated, meaning that a major part of the pipetting is performed by robots. Plate sealings and plate transfers are performed manually.

Actionable protein profiles that are identified in the assays may provide relevant insights into real-time human biology and facilitate development of more effective, targeted therapies. The results are typically used by scientists involved in drug development, clinical research or basic life science research who are looking to run large-scale discovery studies focusing on the low abundant plasma proteome.

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 an introduction to the Olink®Explore platform, including information about the reagents, equipment and documentation needed, an overview of the worfklow, as wells as laboratory guidelines.

The laboratory and data analysis instructions are not described in this manual, refer to *4.1 Olink® documentation*.

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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.Olink® Explore platform

The Olink® Explore platform consists of:

- The following protein biomarker panels:
  - Olink® Explore 384 Cardiometabolic (CARDIO)
  - Olink® Explore 384 Cardiometabolic II (CARDIO II)
  - Olink® Explore 384 Inflammation (INF)
  - Olink® Explore 384 Inflammation II (INF II)
  - Olink® Explore 384 Neurology (NEURO)
  - Olink® Explore 384 Neurology II (NEURO II)
  - Olink® Explore 384 Oncology (ONC)
  - Olink® Explore 384 Oncology II (ONC II)
- Olink® NPX Explore 3072 and NPX™ Map, dedicated softwares for data processing and result reporting.
- Olink® Explore CLI and NPX™ Map CLI

3.Olink® Explore Reagent Kits

The Olink® Explore Reagent Kits can be ordered and run as a set combination of all four or eight panels, a custom combination of four panels, or as a single panel. The kits contain reagents for 96 or 384 samples (including controls).

For information regarding the content of the Olink Explore Reagent Kits, refer to the applicable Olink Explore User Manual. Refer to *4.1 Olink® documentation.*

3.1 Olink® Explore Reagent Kits

Reagent kit	Description
• Olink® Explore 3072 Reagent Kit • Olink® Explore 3072 Reagent Kit (384 samples)	A set combination of eight panels: CARDIO, INF, ONC, NEURO, CARDIO II, INF II, ONC II, NEURO II
• Olink® Explore 4 x 384 Reagent Kit • Olink® Explore 4 x 384 Reagent Kit (384 samples)	A custom combination of four panels among: CARDIO, INF, ONC, NEURO, CARDIO II, INF II, ONC II, NEURO II
Olink® Explore 384 Reagent Kit	One single panel among: CARDIO, INF, ONC, NEURO, CARDIO II, INF II, ONC II, NEURO II

4. Associated documentation

4.1 Olink® documentation

The following Olink® manuals and other resoluts are available from the Olink website: *olink.com/downloads*. As the laboratory workflow differ between Olink Explore Reagent Kits and NGS instruments, each workflow is described in a separate manual. Download the documentation applicable to the Olink Explore Reagent Kit and NGS instrument to be used.

4.1.1 User manuals

Content of the applicable Olink® Explore Reagent Kit and Laboratory instructions:

- Olink® Explore 3072 User Manual
- Olink® Explore 4 x 384 User Manual
- Olink® Explore 384 User Manual

Laboratory instruction to prepare and sequence Olink libraries:

- Olink® Explore Sequencing using NextSeq™ 550
- Olink® Explore Sequencing using NextSeq™ 2000
- Olink® Explore Sequencing using NovaSeq™ 6000
- Olink® Explore 384/3072 Sequencing using NovaSeq™ X Plus

Data processing and analysis of Olink Explore sequence results:

- Olink® NPX Explore 3072 User Manual
- NPX™ Map User Manual
- Olink® Explore CLI Technical Information
- NPX™ Map CLI Technical Information

4.1.2 Other resources

Olink Explore Assay List

- List of all protein assays available in the Olink Explore platform. *olink.com/explore3072/*

Validation data document

- Provides details of the validation results for each individual assay. *olink.com/resources-support/document-download-center/*

Olink guidelines for sample randomization

- Describes the importance of sample randomization and provides guidance on how to perform optimal randomization. *olink.com/faq/sample-randomization*

Safety data sheet

- Plate Control
- Probe Kit
- Sample Prep 1.0–1.3
- Sample Prep 2
- Sample Diluent

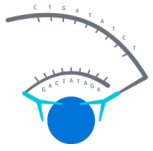
5. Technical support

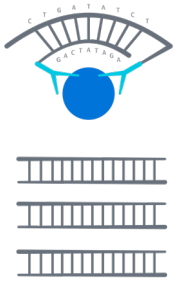
For technical support, contact Olink Proteomics at support@olink.com.

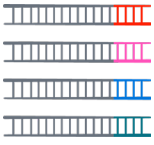
Part 2: Technology description

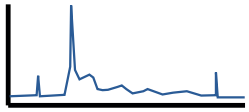
6. About PEA™

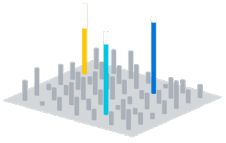
The technology behind the Olink panels is called Proximity Extension Assay (PEA). The Olink Explore platform (PEA with NGS readout) is comprised of a sample preparation that generates an Olink library per panel, followed by quality control and a readout using Next Generation Sequencing (NGS). The process consists of five core steps (the stated durations do not include preparation time):

1. Immuno reaction (Incubation)		Automation steps
Duration of incubation: 16-24h (performed overnight) High-multiplex matched pairs of antibodies, labelled with unique DNA oligonucleotides, bind to their respective proteins in the samples.		**Sample dilution:** – Dragonfly® – F.A.S.T.™ or Mosquito® **Preparation of Incubation Plates:** – F.A.S.T.™ or Mosquito®

2. Extension and pre-amplification (PCR1)		Automation steps
Duration of PCR program: 2h Oligonucleotides that are brought into proximity hybridize and are extended using a DNA polymerase. The piece of DNA barcode that is created is then amplified by a Polymerase Chain Reaction (PCR).		**Preparation of PCR1 Plates:** – Dragonfly® **Pooling of PCR1 products:** – ep*Motion*® or Microlab STAR®

3. Amplification, sample indexing (PCR2) and preparation of Olink libraries		Automation steps
Duration of PCR program: 30 min Unique sample indexes are added to every sample, to allow pooling of the DNA amplicons for all samples. The pooling results in one separate library per panel. Within an Olink library, the final DNA amplicons include: • Specific barcode sequences for each biomarker (or antibody pair) • Sample specific indexes • Required sequences for Illumina sequencing (P5 and P7 Adapters and Sequencing Primer Binding Site Rd1SP)		**Preparation of PCR2 Plate:** – ep*Motion*®, Microlab STAR® or F.A.S.T™ **Pooling of PCR2 products:** – ep*Motion*® or Microlab STAR®

4. Library purification and quality control		AUTOMATION STEPS
Duration of quality control: 30 min Each Olink library is purified using Agentcourt AMPure XP magnetic beads. After this, the library quality is assessed using a Bioanalyzer or Tapestation.		

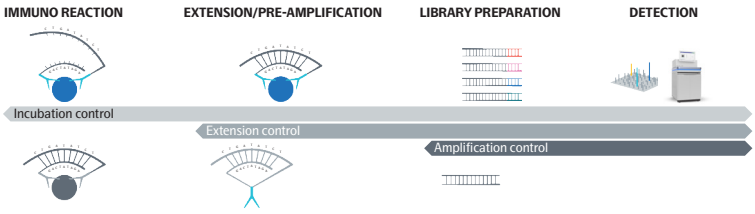
5. NGS readout		AUTOMATION STEPS
Duration of: NextSeq™ 550 run: 7h30 min NextSeq™ 2000 run: 10h30 min NovaSeq™ 6000 run: 7h15 min (SP) or 9h30 min (S4) The Olink library is sequenced by NGS using Illumina® NextSeq™ 550, NextSeq™ 2000 or NovaSeq™ 6000. The relative concentration of each biomarker, based on matched counts[1], is calculated in the Olink high-plex softwares. [1]The number of reads for each specific combination of sample and assay.		

7.Quality control

Olink Explore contains a built-in quality control system using internal and external controls, which enables full control over the technical performance of assays and samples.

7.1 Internal controls

Three internal controls are spiked into every sample for each panel and dilution (the assays for each panel are divided into blocks for optimal read-out quality, and different blocks may have different dilution factors). The internal controls are designed to monitor the quality of assay performance, as well as the quality of individual samples:



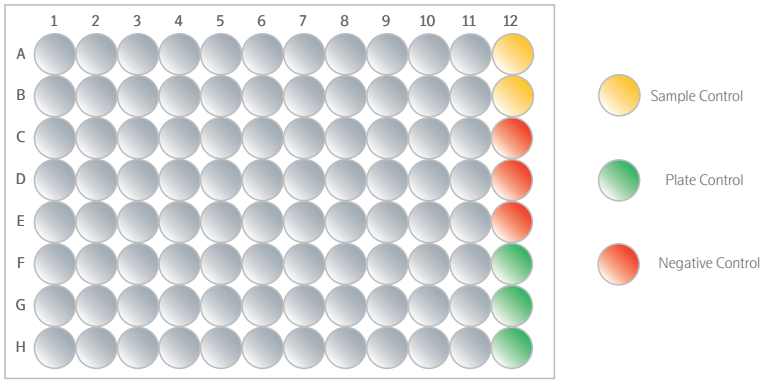
Incubation Control (Immuno Control): The Immuno Control is a non-human antigen measured with PEA. This control is included in the immuno reaction (incubation) and monitors potential technical variation in all three steps of the reaction.

Extension Control: The Extension Control is composed of an antibody coupled to a unique pair of DNA-tags. These DNA-tags are always in proximity, so that this control is expected to give a constant signal independently of the immuno reaction. This control monitors variation in the extension and amplification step.

Amplification Control: The Amplification Control is a complete double stranded DNA amplicon which does not require any proximity binding or extension step to generate a signal. This control monitors the amplification/sample indexing step.

7.2 External controls

External controls are separate samples that are used for different purposes. There are six required and two recommended external controls that are added to separate wells on each sample plate. The figure below shows the sample plate layout, with 88 samples, 2 Sample Controls (optional and supplied by the user), 3 Negative Controls and 3 Plate Controls (required and supplied by Olink).



Sample Control: It is recommended to run a pooled plasma sample in duplicate on each plate. These samples are used to assess potential variation between runs and plates, for example to calculate inter-assay and intra-assay CV, as well as troubleshooting.

Negative Control: Negative Control is included in triplicate on each plate and consists of buffer run as a normal sample. These are used to monitor any background noise generated when DNA-tags come in close proximity without prior binding to the appropriate protein. The Negative Controls set the background levels for each protein assay and are used to calculate the limit of detection (LOD) and to assess potential contamination of assays.

Plate Control: Plate Control is included in triplicate on each plate. The median of the Plate Control triplicates is used to normalize each assay and to compensate for potential variation between runs and plates.

Part 3: Required equipment and consumables

This chapter lists everything that is required to perform an experiment using the Olink Explore protein biomarker platform, excluding the Olink Explore Reagent Kits. Where applicable, it is clearly stated if the items shall be used in the pre-PCR or post-PCR room. This is to facilitate preparation of separate rooms.

For information about the Olink Explore Reagent Kits, refer to *3. Olink® Explore Reagent Kits.*

8.Important information

The Olink Explore protocol has been optimized and validated using the instruments, accessories and consumables listed in this chapter. Comparable performance is not guaranteed when using alternative instruments, accessories, or consumables. In case of support, Olink may be the initial point of contact, but for any hardware related issue, Olink refers to the support of the respective vendors.

9.Software

9.1 Olink high-plex softwares

Olink® NPX Explore 3072 and NPX™ Map are analysis softwares specifically designed for the Olink Explore analysis platform. They differ in the Quality control analysis. Refer to the respective user manuals for further information.

The Olink high-plex softwares come with an accompanying pre-processing software and is required for the generation and analysis of counts files for completed Olink Explore runs.

For further information, refer to the Olink® NPX Explore 3072 User Manual and NPX™ Map User Manual.

9.2 CLI

Olink Explore CLI is a command-line interface (cli) for the Olink Explore products. The application is capable of performing normalization, quality control (QC) and CV computations on NGS data and exporting the results on several supported formats.

For further information, refer to the Olink® Explore CLI Technical Information and NPX™ Map CLI Technical Information.

10.Instruments

This section contains specifications for all instruments and accessories that are required to perform an Olink Explore run. Either one of the instruments marked with the same letter in the figure below described in the associated table can be used.

For detailed instructions regarding the instruments listed in this manual, refer to the documentation provided by the applicable manufacturer.

NOTE: *All robot protocols are subject to changes and registered in different versions (Vx). Before running the experiment, make sure the latest robot protocols are installed.*

Pos	Instrument	Room	Supplier	Article number
A1	**F.A.S.T. Instrument, 96-channel head, transfer range 0.1–13.0 µL**	**Pre-PCR/ Post-PCR**	**Formulatrix**	**814091A**
	– F.A.S.T. Plate adapter for 0.2 mL PCR Strip Tubes, 96 well format.	Pre-PCR/ Post-PCR	Formulatrix	813647A
	– F.A.S.T. Adapter block for semi-skirted and non-skirted PCR plates.	Pre-PCR/ Post-PCR	Formulatrix	815359A
A2	**Mosquito® LV (low volume)**	**Pre-PCR**	**SPT Labtech**	**3019-0036**
	– 5 way Mosquito® Precise Humidity Chamber (PHC)	Pre-PCR	SPT Labtech	3210-01002
	– Mosquito® Application Software	Pre-PCR	SPT Labtech	3019-06101
	– Mosquito® Software licence & comms installed in PC controller	Pre-PCR	SPT Labtech	3019-0030
	– Pipette loader	Pre-PCR	SPT Labtech	3019-03020
	– Pipette tape spool cover	Pre-PCR	SPT Labtech	3019-04134
	– Calibration Block	Pre-PCR	SPT Labtech	3019-05104
	– Magnetic PCI plate clamp **(x 5)**	Pre-PCR	SPT Labtech	3085-01035 / 1x
B	**Dragonfly® discovery 3 head**	**Pre-PCR**	**SPT Labtech**	**3152-10006**
C	**Proflex™ 2 x 384-well PCR System (x 2) Software version 2.0.0 or later NOTE: 2 instruments required**	**Post-PCR**	**Thermo Fisher Scientific**	**4484077 /1x**

Pos	Instrument	Room	Supplier	Article number
D1	**ep*Motion*® 5075lc**	**Post-PCR**	**Eppendorf®**	**5075006019**
	– CleanCap and completely contained housing	Post-PCR	Eppendorf®	5075006019
	– MultiCon PC complete	Post-PCR	Eppendorf®	5075006019
	– TM 50-8 eight-channel dispensing tool, 1–50 µL volume range **(x 2)**	Post-PCR	Eppendorf®	5280000215 / 1x NA: 960001044
	– TM 10-8 eight-channel dispensing tool, 0.2–10 µL **(x 2)**	Post-PCR	Eppendorf®	5280000304 / 1x
	– Reservoir rack	Post-PCR	Eppendorf®	5075754002 NA: 9600002148
	– Thermoadapter for PCR (for temperature control of PCR plates) 384 wells skirted **(x 5)**	Post-PCR	Eppendorf®	5075788004 / 1x NA: 9600002202
D2	**Hamilton Microlab STAR**	**Post-PCR**	**Hamilton**	
	– 8 Channels with 1000µl Pipetting Channels	Post-PCR	Hamilton	173081
	– CO-RE 96 Probehead II 1000ul	Post-PCR	Hamilton	199090
	– Venus Four V4.5 Base Package	Post-PCR	Hamilton	911264-USB
	– Modular Arm for 4 / 8 / 12 Ch. / MPH	Post-PCR	Hamilton	173051
	– Waste Chute, MPH, Left-Side Front	Post-PCR	Hamilton	92573-01
	– Tip Carrier, Landscape (x2-4)	Post-PCR	Hamilton	182085
	– Precision Tab Carrier, MTP, L5 (x1-3)	Post-PCR	Hamilton	93521-01
	– Precision Tab Carrier, DWP, L5	Post-PCR	Hamilton	93522-01
E	**MixMate®**	**Pre-PCR**	**Eppendorf®**	**5353000510 (230V Version)** **5353000529 (110V Version)**
F1	**2100 Bioanalyzer System including chip priming station and IKA Vortex mixer**	**Post-PCR**	**Agilent**	**G2939BA**
F2	**4200 TapeStation System including IKA Vortex mixer**	**Post-PCR**	**Agilent**	**G2991BA**
G1	**NovaSeq™ 6000 Sequencing System**	**Post-PCR**	**Illumina®**	**20012850**
	NovaSeq™ Xp Flow Cell Dock	Post-PCR	Illumina®	20021663
G2	**NextSeq™ 550 Sequencing System**	**Post-PCR**	**Illumina®**	**SY-415-1002**
G3	**NextSeq™ 2000 Sequencing System**	**Post-PCR**	**Illumina®**	**20038897**
G4	**NovaSeq™ X Plus Sequencing System**	**Post-PCR**	**Illumina®**	**20084804**

11.Equipment and consumables: Pre-PCR

This section lists all equipment and consumables required in the pre-PCR room.

11.1 Sample dilution, Incubation and PCR1

Equipment

Equipment	Supplier	Article number
F.A.S.T.™ instrument including accessories	For specifications, refer to *10. Instruments*	
Mosquito® LV including accessories	For specifications, refer to *10. Instruments*	
Dragonfly® discovery 3 head including accessories	For specifications, refer to *10. Instruments*	
Manual pipettes: • 0.5–10 µL • 10–100 µL • 20–200 µL • 100–1000 µL	Any	–
Manual multichannel pipettes (8-channel): 0.5–10 µL	Any	–
Plate centrifuge	Any	–
MixMate® controlled plate vortex**	Eppendorf®	5353000510
Plate vortex	Any	–
Tube vortex	Any	–
Microcentrifuge (high speed not necessary)	Any	–
Pipetboy / Pipette Controller	Any	–
Cooler rack for microcentrifuge tubes	Any	–
Timer	Any	–
Freezing block	Any	–

* Either F.A.S.T. or Mosquito LV can be used.
** A regular plate vortex from any supplier can be used as an alternative to the MixMate®. Refer to *15.1 Vortexing using MixMate®* for more information.

Consumables

Consumables	Supplier	Article number
96-well PCR plate, preferably with full skirt*		–
Filter pipette tips (compatible with manual pipettes)		–
MicroAmp™ Clear Adhesive Film	Thermo Fisher Scientific	4306311 /100x
Plate sealer	Any	–
Twin.tec 384-well PCR plate (skirted)**	Eppendorf®	0030128508 /25x
Sample Control (pooled plasma sample)		
Dragonfly® reservoirs	SPT Labtech	4150-07103
Dragonfly® discovery ultra low retention syringes	SPT Labtech	4150-07208 /100x
F.A.S.T.™ Disposable positive displacement pipette tips (case of 38,400)***		
Spool of Mosquito® pipette tips at 9 mm pitch (26,000 per spool)***	SPT Labtech	4150-03030 /1x
MilliQ water	Any	–
8-well strips with lids	Any	–
50 mL Falcon tubes made of polypropylene	Any	–

* The plates must be able to withstand -80 °C, be dry-ice resistant and easily re-sealable.
** All instrument protocols have been calibrated for this specific plate. Other models should not be used.
***Either F.A.S.T. or Mosquito LV can be used.

12. Equipment and consumables: Post-PCR

This section lists all equipment and consumables required in the post-PCR room.

12.1 Pooling of PCR1 products, PCR2 and pooling of PCR2 products

Equipment

Equipment	Supplier	Article number
2 x ProFlex™ 2 x 384-well PCR System	For specifications, refer to *10. Instruments*	
Formulatrix F.A.S.T.™ including accessories*	For specifications, refer to *10. Instruments*	
ep*Motion*® 5075l including accessories*	For specifications, refer to *10. Instruments*	
Hamilton Microlab STAR® including accessories*	For specifications, refer to *10. Instruments*	
Manual pipettes: • 0.5–10 µL • 10–100 µL • 100–1000 µL	Any	–
Plate centrifuge	Any	–
Plate vortex	Any	–
Tube vortex	Any	–
Microcentrifuge with inserts for both tubes and 8-strip (high speed not necessary)	Any	–
Pipetboy / Pipette Controller	Any	–
Cooler rack for microcentrifuge tubes	Any	–
Freezing block	Any	–

* Either Formulatrix F.A.S.T. , ep*Motion*® 5075lc, or Hamilton Microlab STAR® can be used.

Consumables

Consumables	Supplier	Article number
Twin.tec 384-well PCR plate (skirted)*	Eppendorf®	0030128508 /25x
MilliQ water	Any	–
MicroAmp™ Clear Adhesive Film	Thermo Fisher Scientific	4306311 /100x
Plate sealer	Any	–
15 mL Falcon tube	Any	–
Disposable serological pipettes, 10 mL	Any	–
Filter pipette tips (compatible with manual pipettes)	Any	–
96-well PCR plate, low profile, skirted (for PCR2 pooling)	Thermo Fisher Scientific	AB0800
Microcentrifuge tubes, 1.5 mL	Any	–

* All instrument protocols have been calibrated for this specific plate. Other models should not be used.

F.A.S.T.™ consumables

Consumables	Supplier	Article number
F.A.S.T.™ Disposable positive displacement pipette tips (case of 38,400)*	Formulatrix®	233590

*Either Formulatrix F.A.S.T. or Mosquito LV can be used.

epMotion® consumables

Consumables	Supplier	Article number
ep Dualfilter T.I.P.S.® pipette tips:	Eppendorf®	951020702
• 10 µL		003 0014.391 (with boxes) /10x96 003 0014.553 (refills)
• 50 µL		003 0014.413 (with boxes) /10x96 003 0014.430 (refills)
ep*Motion*® reservoir 30 mL (consumable for each pipetting program)	Eppendorf®	0030 126.505 /10x5
Waste bags bio. for ep*Motion*®, up to 7 L volume	Eppendorf®	5075751763 /50x

Hamilton STAR® consumables

Consumables	Supplier	Article number
50 µL Conductive Filter Tips (Case of 5,760 tips)	Hamilton	235948
300 ml Polystyrene Reservoir, 100 Bulk, Sterile	Integra Biosciences	6328

12.2 Library purification

Equipment

Equipment	Supplier	Article number
DynaMag™-2 Magnet (magnetic stand for Eppendorf® tubes)	Thermo Fisher Scientific	12321D
Manual pipettes: • 10–100 µL • 100–1000 µL	Any	–
Tube vortex	Any	–
Microcentrifuge (high speed not necessary)	Any	–
Timer	Any	–

Consumables

Consumables	Supplier	Article number
Agencourt AMPure XP beads	Beckman Coulter	A63880 / 5 mL
96% Ethanol	Any	–
MilliQ water	Any	–
15 mL Falcon tube	Any	–
Filter pipette tips (compatible with manual pipettes)	Any	–
Disposable serological pipettes • 5 mL • 10 mL	Any	–
Microcentrifuge tubes, 1.5 mL	Any	–

12.3 Quality control of Olink® libraries

Equipment

Equipment	Supplier	Article number
2100 Bioanalyzer System including accessories*	For specifications, refer to *10. Instruments*	
4200 TapeStation System including accessories*	For specifications, refer to *10. Instruments*	
Microcentrifuge (> 13000 x g needed for 2100 Bioanalyzer)	Any	–
Tube vortex	Any	–
Manual pipettes: • 0.5–10 µL • 10–100 µL • 100–1000 µL	Any	–

* Either 2100 Bioanalyzer System or 4200 TapeStation System can be used.

2100 Bioanalyzer consumables

Consumables	Supplier	Article number
Agilent High Sensitivity DNA Kit (includes reagents and 10 chips)	Agilent	5067–4626 /10 chips
Microcentrifuge tubes, 1.5 mL	Any	–
Filter pipette tips (compatible with manual pipettes)	Any	–
MilliQ water	Any	–

4200 TapeStation Consumables

Equipment	Supplier	Article number
High Sensitivity D5000 Reagents	Agilent	5067-5593
High Sensitivity D5000 ScreenTape	Agilent	5067-5592
Loading Tips, 1 Pk	Agilent	5067-5598
Optical tube strip caps (8x Strip)	Agilent	401425
Optical tube strips (8x Strip)	Agilent	401428

12.4 Next generation sequencing using NovaSeq™ 6000

Equipment

Equipment	Supplier	Article number
NovaSeq™ 6000 including accessories	For specifications, refer to *10. Instruments*	
Tube vortex	Any	–
Microcentrifuge (high speed not necessary)	Any	–
Manual pipettes: • 0.5–10 µL • 10–100 µL • 20–200 µL • 100–1000 µL	Any	–
Laboratory bottle 1 L	Any	–
Pipetboy/Pipette Controller	Any	–

Flow cell-specific consumables

Flow cell-specific consumables		Supplier	Article number
SP	NovaSeq™ XP 2-Lane Kit v1.5 (Contains ExAmp reagents and one manifold to load the flow cell)	Illumina	20043130/ 1x
	NovaSeq™ 6000 SP Reagent Kit v1.5 (100 cycles)	Illumina	20028401/ 1x
S4	NovaSeq™ XP 4-Lane Kit v1.5 (Contains ExAmp reagents and one 4-lane manifold to load the flow cell)	Illumina	20043131/ 1x
	NovaSeq™ 6000 S4 Reagent Kit v1.5 (35 cycles)	Illumina	20044417/ 1x

Other consumables

Consumables	Supplier	Article number
Sodium Hypochlorite (NaOCl), 5 % active chlorine, Acros Organics	Thermo Fisher Scientific	419552500/ 250 mL
Tween®20	Sigma-Aldrich	P7949/ 100 mL
Sodium Hydroxide (NaOH), 1.0 N	Sigma-Aldrich	S2770/ 100 mL
Trizma® hydrochloride solution, 1 M (pH 8.0)	Sigma-Aldrich	T2694/ 100 mL
Low linting Wipes (ex. KIMTECH SCIENCE® KIMWIPES™)	Any	–
Used buffer cartridge	Illumina	–
Cluster wash cartridges	Illumina	–
SBS wash cartridges	Illumina	–
MilliQ water, large volumes	Any	–
Filter pipette tips (compatible with manual pipettes)	Any	–
Wash Flow cell or S4 flow cells	Illumina	20016005/ 1x
Microcentrifuge tubes, 1.5 mL	Any	–
Disposable serological pipettes, 5 mL	Any	–

12.5 Next generation sequencing using NextSeq™ 550

Equipment

Equipment	Supplier	Article number
NextSeq™ 550 including accessories	For specifications, refer to *10. Instruments*	
Tube vortex	Any	–
Microcentrifuge (high speed not necessary)	Any	–
Manual pipettes: • 0.5–10 µL • 10–100 µL • 20–200 µL • 100–1000 µL	Any	–

Consumables

Consumables	Supplier	Article number
Sodium Hydroxide (NaOH), 1.0N	Sigma-Aldrich	S2770/ 100 mL
Trizma® hydrochloride solution, 1M pH 7.0	Sigma-Aldrich	T1819/ 100 mL
Lint-free isopropyl alcohol Wipes	Any	–
Low linting Wipes (ex. KIMTECH SCIENCE® KIMWIPES™)	Any	–
NextSeq™ 500/550 High Output Kit v2.5 (75 Cycles)	Illumina	20024906/ 1x
MilliQ water	Any	–
Filter pipette tips (compatible with manual pipettes)	Any	–
Microcentrifuge tubes, 1.5 mL	Any	–

12.6 Next generation sequencing using NextSeq™ 2000

Equipment

Equipment	Supplier	Article number
NextSeq™ 2000 including accessories	For specifications, refer to *10. Instruments*	
Tube vortex	Any	–
Microcentrifuge (high speed not necessary)	Any	–
Manual pipettes: • 0.5–10 µL • 10–100 µL • 20–200 µL • 100–1000 µL	Any	–
Controlled water bath		

Consumables

Consumables	Supplier	Article number
NextSeq™ 1000/2000 P2 Reagents (100 Cycles) v3	Illumina	20046811/ 1x
MilliQ water	Any	–
Filter pipette tips (compatible with manual pipettes)	Any	–
Microcentrifuge tubes, 1.5 mL	Any	–

12.7 Next generation sequencing using NextSeq™ X Plus

Equipment

Equipment	Supplier	Article number
NovaSeq X Plus Sequencing System	For specifications, refer to *10. Instruments*	
Tube vortex	Any	–
Microcentrifuge (high speed not necessary)	Any	–
Manual pipettes: • 0.5–10 µL • 10–100 µL • 20–200 µL • 100–1000 µL	Any	–

Flow cell specific consumables

Consumables		Supplier	Article number
1.5B	NovaSeq X Series 1.5B Reagent Kit (100 cycles)	Illumina	20104703
10B	NovaSeq X Series 10B Reagent Kit (100 cycles)	Illumina	20085596

Other consumables

Consumables	Supplier	Article number
Sodium Hypochlorite (NaOCl), 5 % active chlorine, Acros Organics	Thermo Fisher Scientific	419552500/ 250 mL
Sodium Hydroxide (NaOH), 1.0 N	Sigma-Aldrich	S2770/ 100 mL
Contec Polynit Heatseal wipes	VWR	68310-176
MilliQ water, large volumes	Any	–
Filter pipette tips (compatible with manual pipettes)	Any	–
Microcentrifuge tubes, 1.5 mL	Any	–
Reagent or spectrophotometric-grade isopropyl alcohol (70%), 100 ml bottle	Any	–

Part 4: Guidelines

13.Safety considerations

13.1 Safety instructions

Follow general laboratory safety procedures:

- Use gloves, safety goggles and protective clothing when performing the experiments.
- Handle solutions with particular caution, as Dimethyl sulfoxide (DMSO) is known to facilitate the entry of organic molecules into tissues.
- Handle and dispose of hazardous sample material according to local regulations.

For complete safety information, refer to Material Safety Data Sheets (MSDS) available on the Olink website: *www.olink. com/downloads*.

13.2 Laboratory setup

The high sensitivity of the Olink assays requires a clean laboratory environment. Particles from the surroundings, such as dust, hair, saliva and skin flakes are common sources of contamination.

A well-recognized risk with molecular detection methods is contamination from PCR products. Therefore, make sure to organize the workspace so that the workflow occurs in one direction: from clean areas free from PCR products (pre-PCR) to areas containing PCR products (post-PCR). Olink recommends setting up at least two separate rooms: one pre-PCR and one post-PCR room. If this is not possible, keep separate benches and equipment.

13.3 Clean laboratory environment

The following recommendations are intended to reduce the risk of contamination, simplify the workflow in the laboratory and improve data quality. Make sure to follow these recommendations at all times.

- Use separate rooms for pre-PCR and post-PCR operations.
- Use separate consumables and equipment for pre-PCR and post-PCR operations.
- Always work from clean areas free from PCR products (pre-PCR) to areas containing PCR products (post-PCR).
- Fit ultra-violet (UV) lamps in closed working areas such as working cabinets or pipetting robots to enable decontamination by irradiation.
- Always wear a long-sleeved lab coat.
- Always wear gloves, including when bringing reagents in and out from fridge or freezer. Change gloves when needed.
- Wash your hands and change gloves and lab coat when moving between pre-PCR and post-PCR.
- Regularly decontaminate bench spaces with 10 % sodium hypochlorite (followed by water to remove residual bleach), or a validated commercially available DNA-degrading decontaminant.
- Decontaminate pipettes on a regular basis according to the manufacturer's instructions.
- Clean instruments and pipetting robots on a regular basis according to the manufacturer's instructions.
- Keep all consumables (tubes, pipette tips, PCR plates etc.) in closed bags or boxes, preferably in a closed storage unit, until use.
- Clean the lab bench, hood, racks and pipettes with 70 % ethanol.
- Bring out all reagents, consumables and samples needed for the specific lab step, as stated in the "Prepare the bench" list at the beginning of each instruction. Leave enzymes in the freezer until use.
- Organize equipment, consumables and samples at the workstation in a way that enables clean work.
- Label pipette boxes with column numbers to more easily monitor where you are on the plate.
- Briefly centrifuge tubes and plates before opening to avoid the generation of aerosols that may contaminate other samples.
- Pipette all reagents and samples using filter tips, and use a unique set of pipettes for each working station.

NOTE: *All consumables and reagents are for single use only.*

14. Pipetting techniques

Both forward and reverse pipetting are used in the Olink Explore workflow. Forward pipetting is the most commonly used pipetting technique. Reverse pipetting improves precision with smaller volumes and viscous solutions. Both techniques are described in this section, along with general guidelines for pipetting.

14.1 General pipetting guidelines

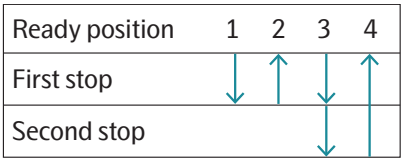
- Calibrate all pipettes regularly (at least with a 6-month interval).
- Let the reagents and liquids reach room temperature before use to maximize accuracy.
- Pipette near the liquid surface.
- Do not turn the pipette on the side when there is liquid in the tip, as liquid might contaminate the interior of the pipette.
- Keep the pipettes vertical while pipetting, and pipette to the bottom of the wells.

14.2 Forward pipetting

1. Press the operating button to the first stop.
2. Dip the tip into the solution to a depth in accordance with the set volume, and slowly release the operating button. Remove the tip from the liquid.

NOTE: *If using a multichannel pipette, ensure that all tips contain the exact same volume.*

3. Dispense the liquid into the receiving vessel by gently pressing the operating button to the first stop and then to the second stop. This action will empty the tip. Remove the tip from the vessel.
4. Release the operating button to the ready position.



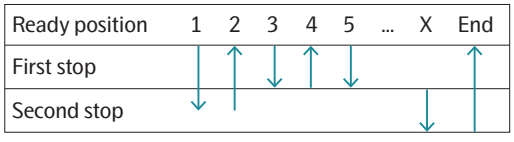
14.3 Reverse pipetting

When using reverse pipetting, pre-rinse the tip 1 to 3 times with the liquid to be pipetted to improve accuracy.

1. Press the operating button past the first stop.
2. Dip the tip into the solution to a depth in accordance with the set volume, and slowly release the operating button. This action will fill the tip with a volume that is larger than the set volume.

NOTE: *If using a multichannel pipette, ensure that all tips contain the exact same volume.*

3. Remove the tip from the liquid and dispense the liquid into the receiving vessel by pressing the operating button gently and steadily to the first stop. The dispensed volume is equal to the set volume.
4. Hold the button in this position. Some liquid will remain in the tip, which should not be dispensed.
5. Continue pipetting by repeating steps 3 and 4.



15.Plate vortexing

Correct vortexing is essential to generate reproducible results when running Olink Explore Reagent Kits. For best results, the Eppendorf MixMate® controlled plate vortex should be used to vortex plates during pre-PCR1 steps. When using manual vortexing, use the manual vortexing technique described below to ensure thorough vortexing of the plates content.

15.1 Vortexing using MixMate®

1. Insert the plate into the plate holder by placing it at the rear of the plate holder and pressing it down until it is firmly seated. Note that misplacement of the plate will result in uneven mixing of the wells and low-quality data.
2. Set mixing speed and time according to:

Plate type	Speed (rpm)	Time (s)
96-wells	2500	30
384-wells	3000	30

IMPORTANT: *make sure to use the correct MixMate settings as incorrect settings may lead to low-quality data.*

3. Start mixing by pressing the start/stop key. After 30 seconds, MixMate will automatically stop.
4. Take out the plate by pulling it out from the front side of the plate holder.

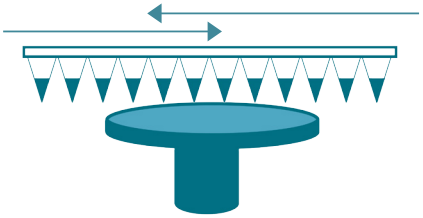
15.2 Manual vortexing

A manual vortex is needed to vortex all separate tubes throughout the protocol. A manual vortex may also be used for the vortexing of plates during the detection step, if so, follow the below instructions.

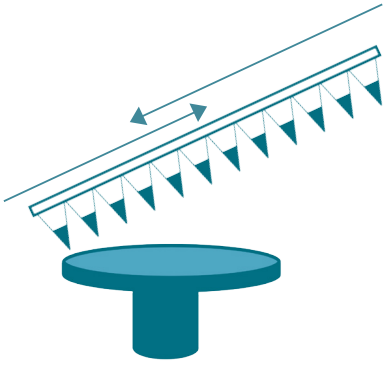
- When using a manual vortex, cover the rubber platform of the vortex with adhesive plastic film to make it easier to slide the 96-well plate during vortexing.
- Vortex for 20–30 seconds at full speed.
- Visually inspect the wells during vortexing to ensure complete mixing. The liquid should swirl in the wells.

Instructions

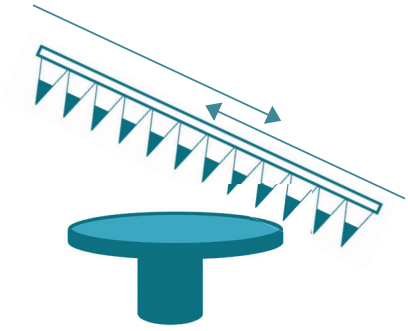
1. Move the plate back and forth over the vortex in a horizontal direction. Make sure that the wells at the outside edges of the plate are also vortexed.



2. Tilt the plate away from you and move it back and forth over the vortex.



3. Tilt the plate towards you and move it back and forth over the vortex.



4. Turn the plate 180° and repeat steps 1–3.

You can also see our vortexing *video* for further guidance on manual vortexing.

NOTE: *Cover the rubber platform of the vortex with adhesive film to make it easier to slide the plates during vortexing.*

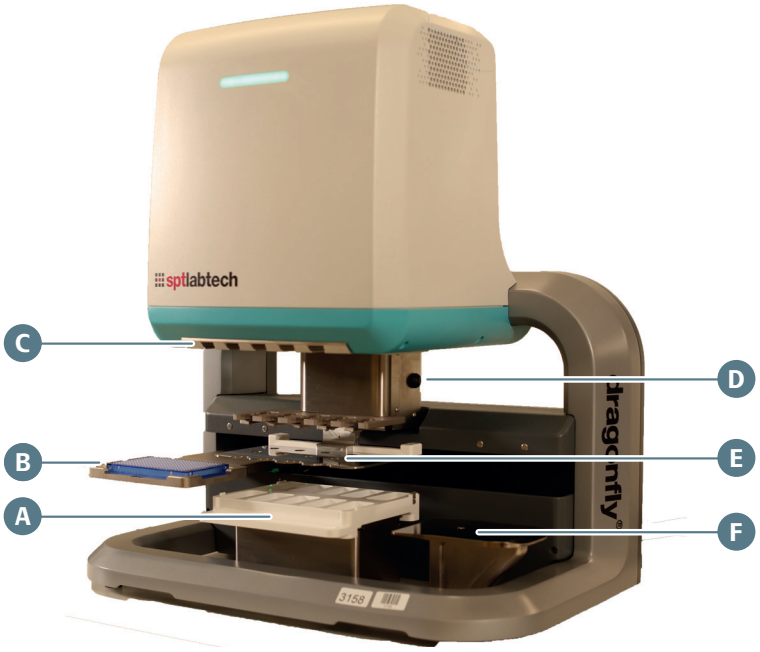
16. Plate centrifugation

Common centrifuges only have the speed setting in RPM. As the centrifugation force depends on the size of the rotor, the corresponding g force will vary between different centrifuges. Contact the centrifuge vendor to get help converting RPM to g.

17. Using the Dragonfly®

This section covers the tasks most commonly performed on the Dragonfly during the Olink Explore workflow. For detailed instrument instructions, refer to the instrument's user manual.

17.1 Dragonfly® overview



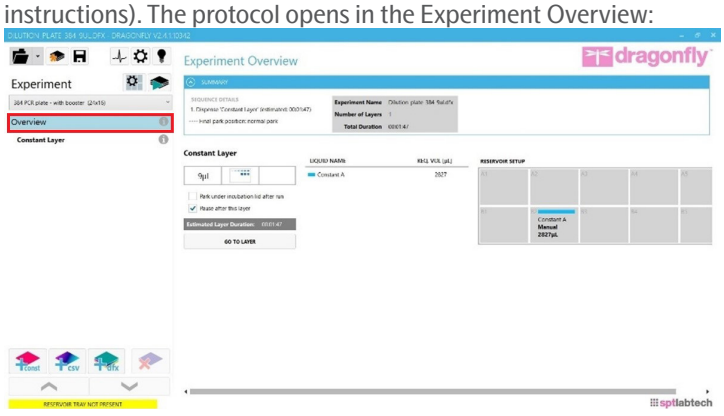
Position	Description
A	Aspirate position (with reservoir tray in place)
B	Plate position (with plate in place)
C	Syringe plate
D	Button for lowering the syringe guard
E	Syringe guard (in upper position)
F	Fill position

17.2 Lower and raise the Dragonfly® syringe guard

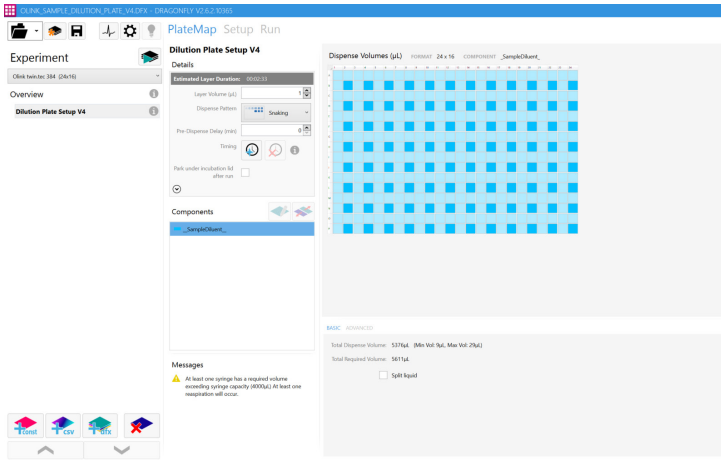
- To lower the syringe guard, push the black button on the right side of the instrument.
- To raise the syringe guard, push it upwards manually until it clicks.

17.3 Prepare the Dragonfly®

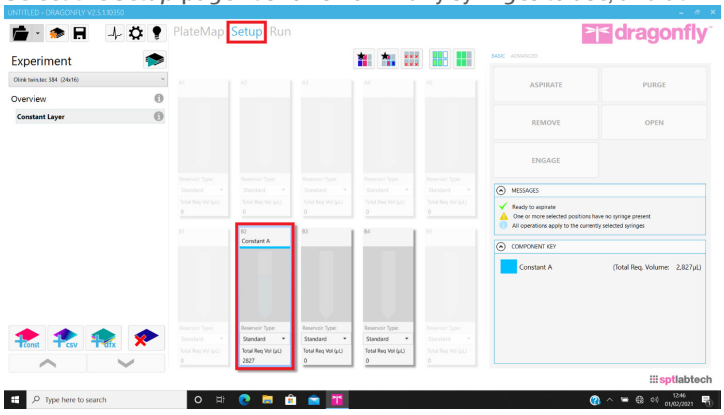
1. Switch on the Dragonfly and the computer. Make sure that the syringe guard is in the upper position and that the reservoir tray is in the aspirate position.
2. Wait at least 30 seconds before opening the Dragonfly software .
3. Click on the folder icon at the top left corner and open the applicable protocol (according to instructions). The protocol opens in the Experiment Overview:



4. Select *Constant Layer* to show the *Plate Map* with the layout of the applicable plate:

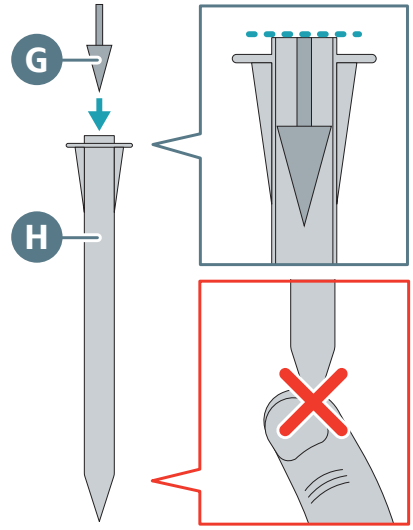


5. Select the *Setup* page. It shows how many syringes to use, and at which positions they shall be inserted.



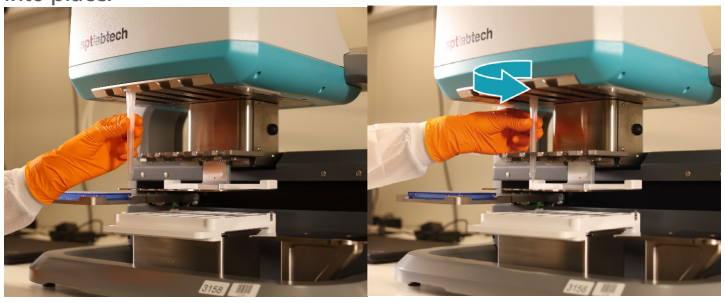
6. Lower the syringe guard.

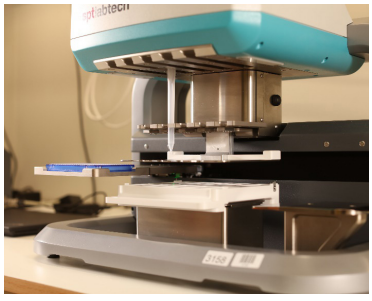
7. Carefully insert a plunger (G) into a new disposable syringe (H) and push it down until flush with the upper edge of the syringe.

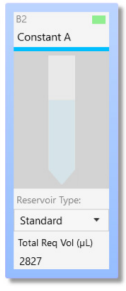


NOTE: *Be careful not to touch or damage the syringe tip.*

8. Attach the disposable syringe including plunger to the applicable track of the syringe plate and slide it in until it reaches the applicable groove. Rotate the syringe 90 degrees counterclockwise until it clicks into place.







NOTE: *Successful insertion of the syringe is indicated by a green symbol at the corresponding syringe position in the Setup page.*

9. Repeat steps 7–8 as necessary if more than one syringe is required.
10. Raise the syringe guard manually to the upper position.

17.3.1 Shut the Dragonfly® down

1. Make sure that the reservoir tray is at the aspirate position, with the disposable reservoir placed beneath the syringe.
2. In the *Setup* page of the software, click *REMOVE* to disengage the syringe.

IMPORTANT: *Do not try to remove the syringe while it is still engaged, as this might damage the instrument.*

3. When the syringe is disengaged, push the black button on the right side of the instrument to lower the syringe guard.
4. Turn the syringe 90 degrees clockwise, then slide it out from the instrument. Discard the syringe including the plunger.
5. Slide the reservoir tray to the right until it reaches the filling position. Remove the used disposable reservoir and discard it.
6. Raise the syringe guard manually.
7. Click on the *X* button in the upper right corner of the window to shut down the software.
8. Switch off the Dragonfly and the computer.

18. Using the Mosquito®

This section covers the tasks most commonly performed on the Mosquito during the Olink Explore workflow. For detailed instrument instructions, refer to the instrument's user manual.

18.1 Mosquito® overview

Position	Description
A	LED Light
B	Plate deck
C	Humidifier chambers

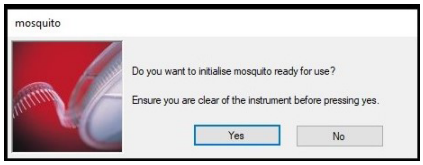
18.2 Prepare the Mosquito®

1. Turn on the Mosquito using the on/off switch at the back of the instrument. The LED light around the LV sign at the top left of the instrument lights up when it is ready to run.

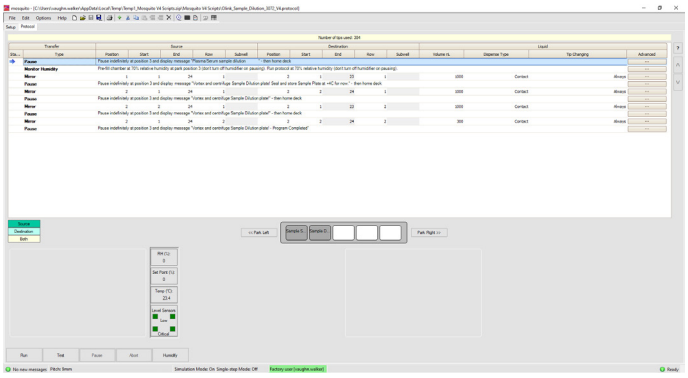
2. The software will warn the user to refill the reservoirs at low level as well as at critical level of water. If the level is critical the system will not run. To refill the reservoirs, open the Perspex cover and then lift the lid off the humidifier by placing one or two fingers in the oval slot and lifting the lid off. Refill the reservoirs with MilliQ water up to the High-Level Indicator “H”. Then place the lid back on.

Humidifier chamber with lid on (left). Humidifier chamber with the lid off (right) indicating how to refill water in reservoir.

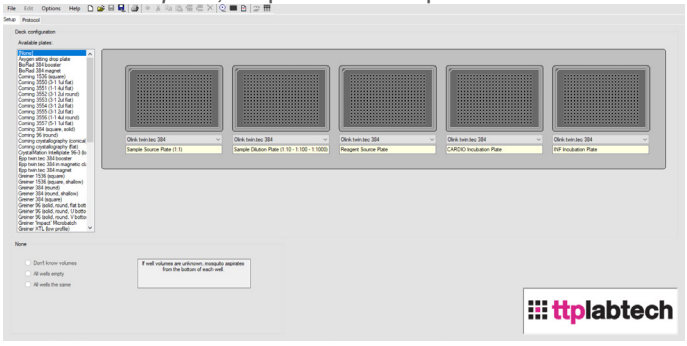
3. Wait at least 30 seconds before turning on the computer and opening the Mosquito software .
4. When a prompt asks if you want to initialize the instrument, make sure that the Plate deck is empty and click *Yes*.



5. Select *File/Open* and choose the applicable protocol (according to instructions) from the Load Protocol Window.
   - Under the *Protocol* tab, the different steps in the protocol are listed. Some steps contain instructions that are shown on the screen during the run. The humidity display will change color depending on the humidity (blue within ±5 % of the Set Point and red when outside ±5 % of the Set Point).



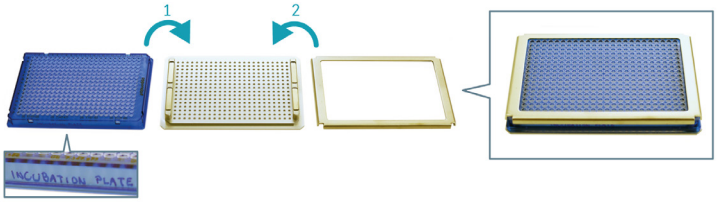
   - Under the *Setup* tab, the position of the plates is shown:



NOTE: *All plates that are included in the protocol are listed, but they may not all be used at the same time. Make sure to follow the instructions for the applicable protocol, adding/removing plates as described during the protocol run.*

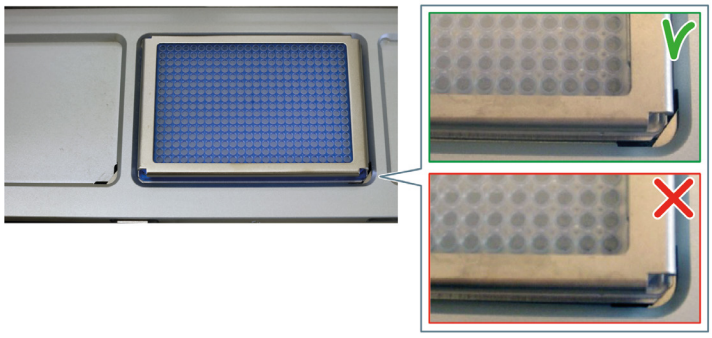
18.3 Using a magnetic clamp booster in Mosquito®

6. Place the applicable plate in a magnetic clamp booster. Numbers and letters on the plate will not be visible in the magnetic clamp booster, so note which way the plate is turned.



NOTE: *Mark the plate on the side without letters.*

7. Place the magnetic clamp booster including the plate in the correct position (shown in the software) on the Mosquito deck. Insert the corners of the magnetic clamp booster under the small metal holders of the deck as shown in the figure below. This applies to all plate positions of the Mosquito deck.



IMPORTANT: *Risk of instrument damage! If the magnetic clamp booster is not correctly placed it may collide with the internal parts of the instrument during the run.*

19.Revision history

Version	Date	Description
5.0	2025-01-27	Changed name of NPX Explore to NPX Map.
4.1	2024-07-08	*6* replaced Olink MyData Cloud software with NPX™ Explore software
4.0	2024-04-16	*4.1* updated. *9.1* updated, *9.2* added. *10* and *12.1* updated: F.A.S.T. as post-PCR instrument *10* and *12.7* updated: NovaSeq X Plus as sequencing instrument. *15.1* Important added Editorial changes
3.0	2023-03-16	New trademarks and disclaimer. *17.3*, step 4: figure updated. *18.2*, step 4: figure updated.
2.0	2023-02-16	Olink® Explore Expansion removed. Tapestation added. *10* figure and table updated. *12.3,* equipment table and Tapestation table updated.
1.3	2022-12-21	*10:* figure and table updated. Former table 8 split into three consumables tables. Hamilton STAR® added. Name change of Certificate of Analysis to Analysis Report.
1.2	2022-05-13	*10:* table - changed MixMate® article number. *Table 5* added freezing block. *Table 7* added freezing block. *18.2*, step 2: edited instrument position and figure legend. *18.2* edited steps 1, 2, and 3.
1.1	2021-12-13	*10:* table - changed information about MixMate®. *Table 6* added information for Falcon tubes.
1.0	2021-12-01	New

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1187, v5.0, 2025-01-27