# Olink® Explore Overview

## User Manual

1187, 4.1, 2024-07-10

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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*.



### 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 3076 and NPX™ Explore HT & 3072, dedicated softwares for data processing and result reporting.
- Olink® Explore CLI and NPX™ Explore CLI HT & 3072

# 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<br>• Olink® Explore 3072 Reagent Kit (384 samples) | A set combination of eight panels:<br>CARDIO, INF, ONC, NEURO, CARDIO II, INF II, ONC II, NEURO II |
| • Olink® Explore 4 x 384 Reagent Kit<br>• Olink® Explore 4 x 384 Reagent Kit (384 samples) | A custom combination of four panels among:<br>CARDIO, INF, ONC, NEURO, CARDIO II, INF II, ONC II, NEURO II |
| Olink® Explore 384 Reagent Kit | One single panel among:<br>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™ Explore HT & 3072 User Manual
- Olink® Explore CLI Technical Information
- NPX™ Explore CLI HT & 3072 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

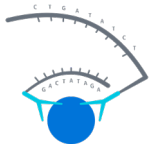
# 5. Technical support

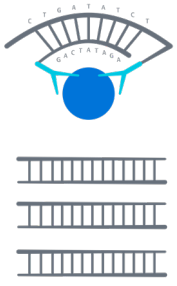
For technical support, contact Olink Proteomics at support@olink.com.

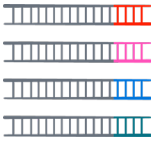
# Part 2: Technology description

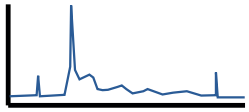
# 6. About PEA™

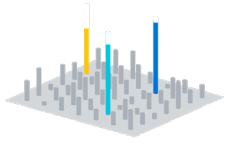
The technology behind the Olink panels is called Proximity Extension Assay (PEA). The Olink Explore platform (PEA with NGS readout) is comprised of a sample preparation that generates an Olink library per panel, followed by quality control and a readout using Next Generation Sequencing (NGS). The process 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)<br><br>High-multiplex matched pairs of antibodies, labelled with unique DNA oligonucleotides, bind to their respective proteins in the samples. |  | **Sample dilution:**<br>– Dragonfly®<br>– F.A.S.T.™ or Mosquito®<br>**Preparation of Incubation Plates:**<br>– F.A.S.T.™ or Mosquito® |

| 2. Extension and pre-amplification (PCR1) | | Automation steps |
|---|---|---|
| Duration of PCR program: 2h<br><br>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:**<br>– Dragonfly®<br>**Pooling of PCR1 products:**<br>– ep*Motion*® or Microlab STAR® |

| 3. Amplification, sample indexing (PCR2) and preparation of Olink libraries | | Automation steps |
|---|---|---|
| Duration of PCR program: 30 min<br><br>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.<br><br>Within an Olink library, the final DNA amplicons include:<br>• Specific barcode sequences for each biomarker (or antibody pair)<br>• Sample specific indexes<br>• Required sequences for Illumina sequencing (P5 and P7 Adapters and Sequencing Primer Binding Site Rd1SP) |  | **Preparation of PCR2 Plate:**<br>– ep*Motion*®, Microlab STAR® or F.A.S.T™<br>**Pooling of PCR2 products:**<br>– ep*Motion*® or Microlab STAR® |

| 4. Library purification and quality control | | Automation steps |
|---|---|---|
| Duration of quality control: 30 min<br><br>Each Olink library is purified using Agentcourt AMPure XP magnetic beads. After this, the library quality is assessed using a Bioanalyzer or Tapestation. |  | |

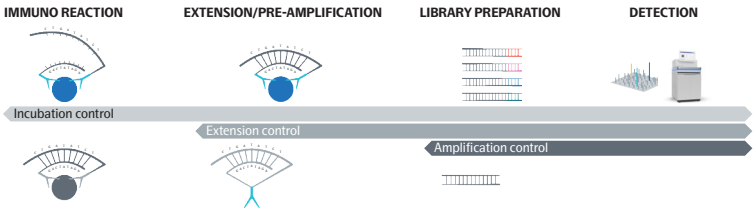
| 5. NGS readout | | Automation steps |
|---|---|---|
| Duration of:<br>NextSeq™ 550 run: 7h30 min<br>NextSeq™ 2000 run: 10h30 min<br>NovaSeq™ 6000 run: 7h15 min (SP) or 9h30 min (S4)<br><br>The Olink library is sequenced by NGS using Illumina® NextSeq™ 550, NextSeq™ 2000 or NovaSeq™ 6000. The relative concentration of each biomarker, based on matched counts[1], is calculated in the NPX™ Explore software.<br><br>[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:



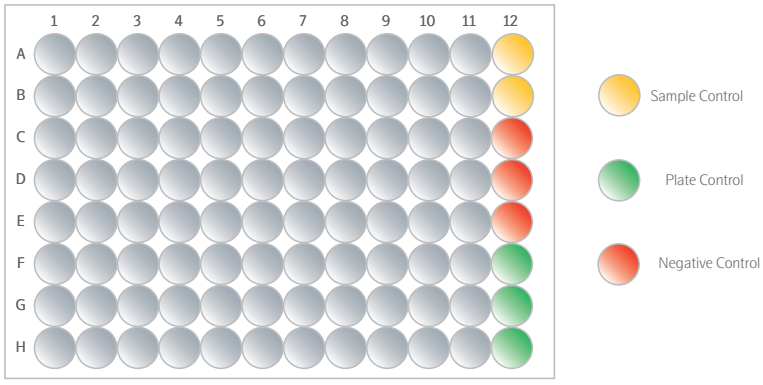

**Incubation Control (Immuno Control):** The Immuno Control is a non-human antigen measured with PEA. This control is included in the immuno reaction (incubation) and monitors potential technical variation in all three steps of the reaction.

**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 NPX Explore softwares

Olink® NPX Explore 3072 and NPX™ Explore HT & 3072 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 NPX™ Explore HT & 3072 comes 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™ Explore HT & 3072 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™ Explore CLI HT & 3072 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<br>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<br>NA: 9600002148 |
| | – Thermoadapter for PCR (for temperature control of PCR plates) 384 wells skirted **(x 5)** | Post-PCR | Eppendorf® | 5075788004 / 1x<br>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)**<br>**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:<br>• 0.5–10 µL<br>• 10–100 µL<br>• 20–200 µL<br>• 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:<br>• 0.5–10 µL<br>• 10–100 µL<br>• 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<br>003 0014.553 (refills) |
| • 50 µL | | 003 0014.413 (with boxes) /10x96<br>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:<br>• 10–100 µL<br>• 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<br>• 5 mL<br>• 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:<br>• 0.5–10 µL<br>• 10–100 µL<br>• 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:<br>• 0.5–10 µL<br>• 10–100 µL<br>• 20–200 µL<br>• 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:<br>• 0.5–10 µL<br>• 10–100 µL<br>• 20–200 µL<br>• 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:<br>• 0.5–10 µL<br>• 10–100 µL<br>• 20–200 µL<br>• 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:<br>• 0.5–10 µL<br>• 10–100 µL<br>• 20–200 µL<br>• 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

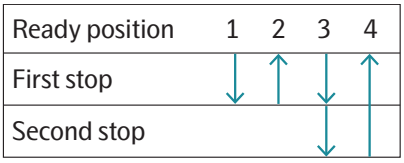
- Calibrate all pipettes regularly (at least with a 6-month interval).
- Let the reagents and liquids reach room temperature before use to maximize accuracy.
- Pipette near the liquid surface.
- Do not turn the pipette on the side when there is liquid in the tip, as liquid might contaminate the interior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



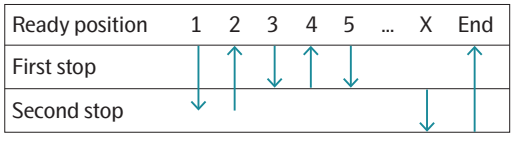

## 14.3 Reverse pipetting

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.

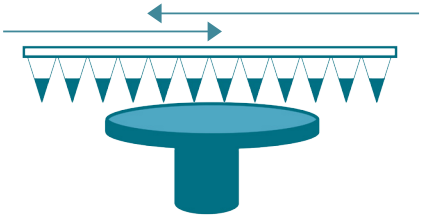
## 15.2 Manual vortexing

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.

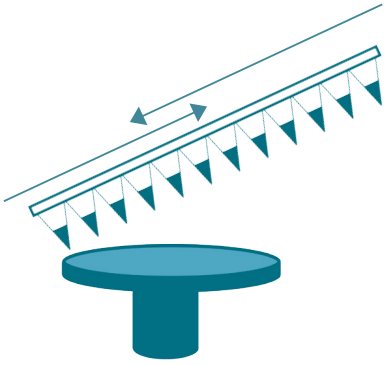
- When using a manual vortex, cover the rubber platform of the vortex with adhesive plastic film to make it easier to slide the 96-well plate during vortexing.
- Vortex for 20–30 seconds at full speed.
- Visually inspect the wells during vortexing to ensure complete mixing. The liquid should swirl in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

**Instructions**

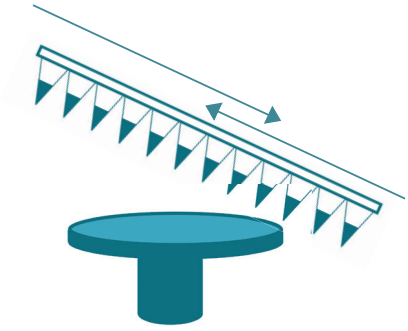
1. Move the plate back and forth over the vortex in a horizontal direction. Make sure that the wells at the outside edges of the plate are also vortexed.



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.*

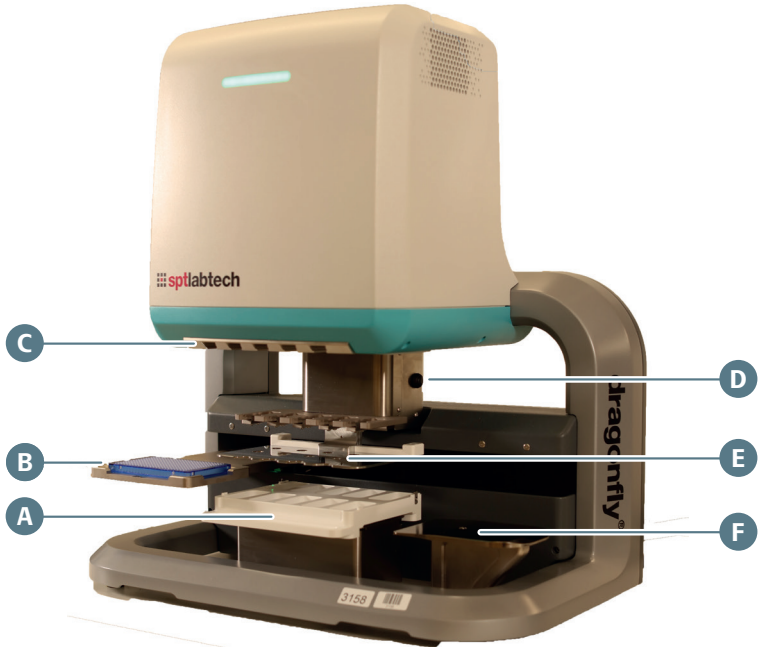
# 16. Plate centrifugation

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



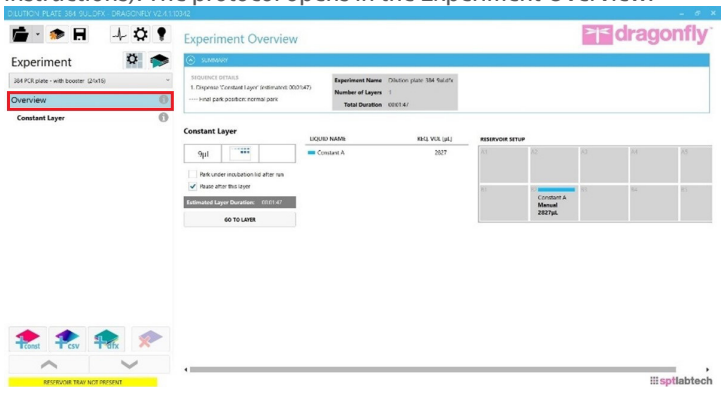

| Position | Description |
|---|---|
| **A** | Aspirate position (with reservoir tray in place) |
| **B** | Plate position (with plate in place) |
| **C** | Syringe plate |
| **D** | Button for lowering the syringe guard |
| **E** | Syringe guard (in upper position) |
| **F** | Fill position |

## 17.2 Lower and raise the Dragonfly® syringe guard

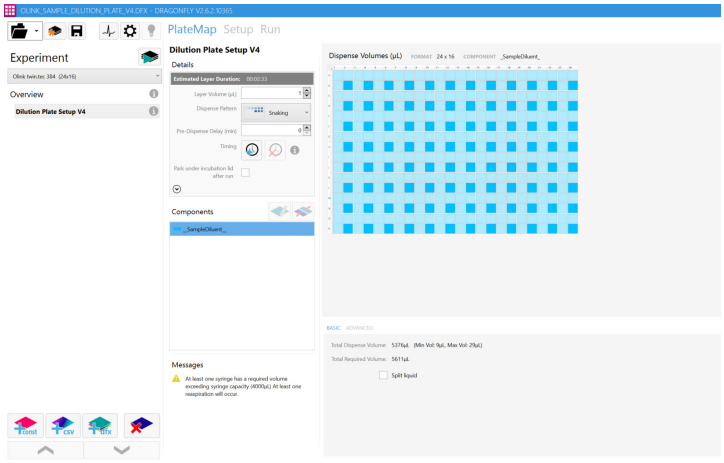
- To lower the syringe guard, push the black button on the right side of the instrument.
- To raise the syringe guard, push it upwards manually until it clicks.

## 17.3 Prepare the Dragonfly®

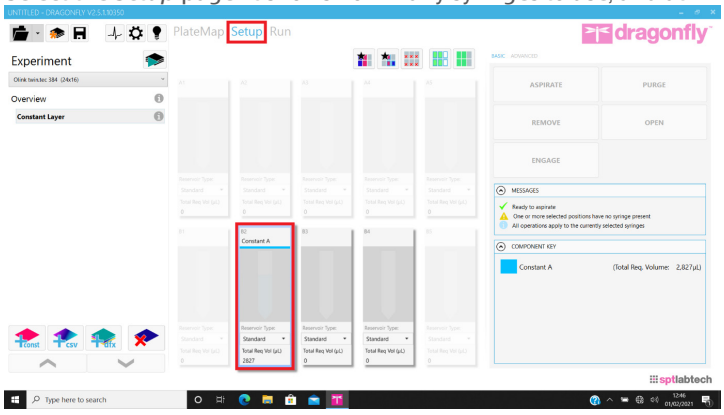
1. Switch on the Dragonfly and the computer. Make sure that the syringe guard is in the upper position and that the reservoir tray is in the aspirate position.
2. Wait at least 30 seconds before opening the Dragonfly software .
3. Click on the folder icon at the top left corner and open the applicable 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:

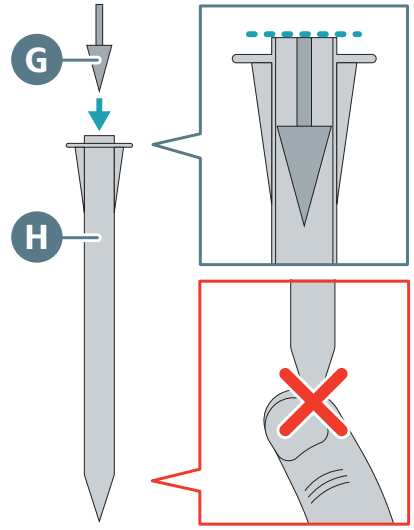



5. Select the *Setup* page. It shows how many syringes to use, and at which positions they shall be inserted.



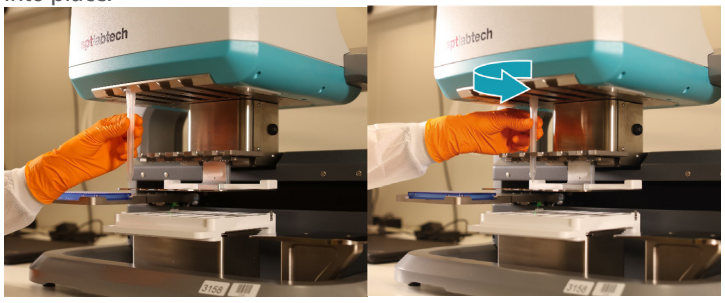

6. Lower the syringe guard.

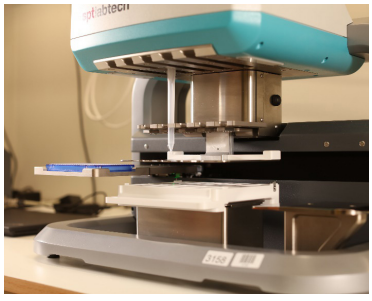
7. Carefully insert a plunger (G) into a new disposable syringe (H) and push it down until flush with the upper edge of the syringe.

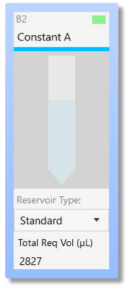



**NOTE:** *Be careful not to touch or damage the syringe tip.*

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 |

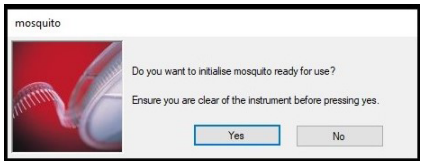
## 18.2 Prepare the Mosquito®

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.

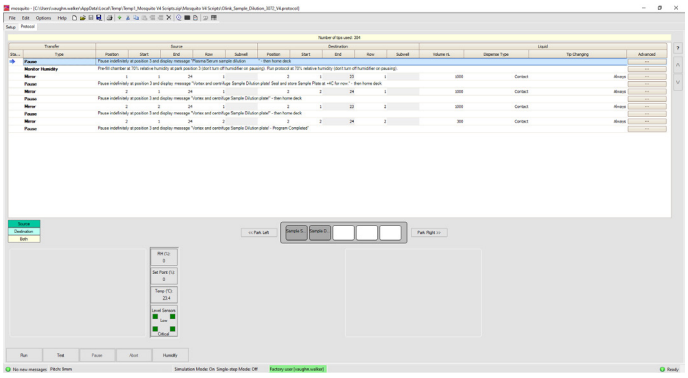
2. The software will warn the user to refill the reservoirs at low level as well as at critical level of water. If the level is critical the system will not run. To refill the reservoirs, open the Perspex cover and then lift the lid off the humidifier by placing one or two fingers in the oval slot and 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.

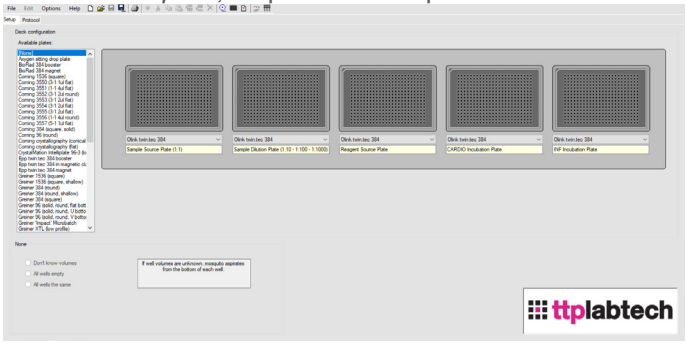
3. Wait at least 30 seconds before turning on the computer and opening the Mosquito software .
4. When a prompt asks if you want to initialize the instrument, make sure that the Plate deck is empty and click *Yes*.




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).



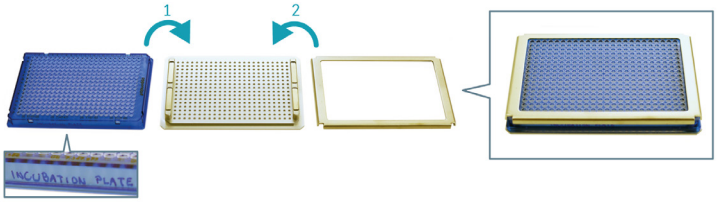
   - Under the *Setup* tab, the position of the plates is shown:




**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.*

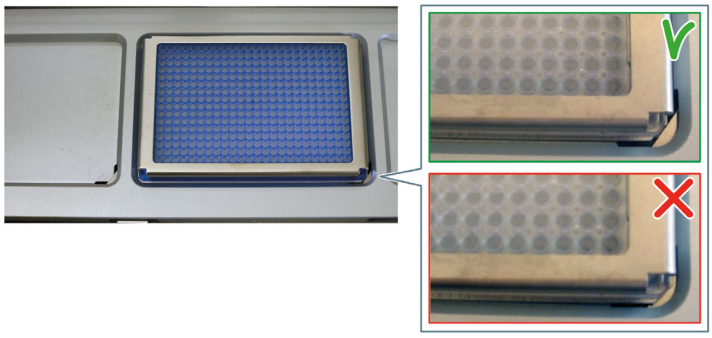
## 18.3 Using a magnetic clamp booster in Mosquito®

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

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