Olink® Explore HT overview

User Manual

1293, v4.0, 2024-10-07

Document note

The Olink® Explore HT User Manual, doc no 1293, has been divided into three manuals:

- Olink® Explore HT Overview User Manual, doc no 1293
- Olink® Explore HT Formulatrix® F.A.S.T.™ Laboratory Instructions, doc no 1548
- Olink® Explore HT SPT Labtech Mosquito® Laboratory instructions, doc no 1549

Table of contents

1.Introduction

Olink® Explore HT 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 most pipetting steps are performed by robots. Plate sealings and plate transfers are performed manually.

Actionable protein profiles that are identified by 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. 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 Olink® Explore HT, including information about reagents, equipment and documentation needed, an overview of the workflow, laboratory guidelines as well as instructions needed to run the Olink® Explore HT Reagent Kit.

For optimal results, the instructions must be strictly and explicitly followed. Any deviations throughout the laboratory steps may result in impaired data.

1.3 Definition of alert levels

The following alert levels are used in the Olink Explore HT manuals:

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 HT Reagent Kit contents

Olink® Explore HT Reagent Kit contains reagents for 172 user samples and 20 Olink controls spread over two sample plates. Storage temperature and expiry date for the components are stated on the label on each box.

2.1 Content of Olink® Explore HT Probes (98001)

Art. No	Component	Cap color	Volume	Storage
88030	Olink® Explore HT Frw Probes 1	White	25 uL	+4 ºC
88031	Olink® Explore HT Rev Probes 1	White	25 uL	
88032	Olink® Explore HT Frw Probes 2	Red	25 uL	
88033	Olink® Explore HT Rev Probes 2	Red	25 uL	
88034	Olink® Explore HT Frw Probes 3	Yellow	25 uL	
88035	Olink® Explore HT Rev Probes 3	Yellow	25 uL	
88036	Olink® Explore HT Frw Probes 4	Blue	25 uL	
88037	Olink® Explore HT Rev Probes 4	Blue	25 uL	
88038	Olink® Explore HT Frw Probes 5	Green	25 uL	
88039	Olink® Explore HT Rev Probes 5	Green	25 uL	
88040	Olink® Explore HT Frw Probes 6	Purple	25 uL	
88041	Olink® Explore HT Rev Probes 6	Purple	25 uL	
88042	Olink® Explore HT Frw Probes 7	Orange	25 uL	
88043	Olink® Explore HT Rev Probes 7	Orange	25 uL	
88044	Olink® Explore HT Frw Probes 8	Black	25 uL	
88045	Olink® Explore HT Rev Probes 8	Black	25 uL	
88003	Olink® Explore HT PCR Additive	Brown	4000 uL	
87004	Olink® Explore Incubation solution	Brown	1400 uL	

2.2 Content of Olink® Explore HT Sample Prep (98023)

Art. No	Component	Cap color	Volume	Storage
88001	Olink® Explore HT PCR Solution	Natural	4000 µL	-20 ºC
88006	Olink® Explore HT PCR Enzyme A	Blue	825 µL	
88007	Olink® Explore HT PCR Enzyme B	Violet	115 µL	
87009	Olink® Explore Negative Control	Red	150 µL	
88016	Olink® Explore Index Plate C	Green	15 µL	
84032	Olink® Target 96/Explore Sample Diluent	Natural	2x13 mL	

2.3 Content of Olink® Explore HT Controls (98003)

Art. No	Component	Cap color	Volume	Storage
87010	Olink® Explore Plate Control	Green	160 µL	-80 ºC
88011	Olink® Explore Sample Control	Yellow	90 µL	

3. Associated documentation and resources

3.1 Olink documentation

Laboratory Instructions

- Olink® Explore HT Formulatrix F.A.S.T.™ Laboratory Instructions
- Olink® Explore HT SPT Labtech Mosquito® Laboratory Instructions

User Manuals

- Olink Explore HT Sequencing using NovaSeq 6000 S4
- Olink Explore HT Sequencing using NovaSeq X Plus
  - For sequencing of Olink Explore HT libraries
- NPX™ Explore HT & 3072 User Manual
- NPX™ CLI Explore HT & 3072 Technical Information
  - For data analysis of Olink Explore HT libraries

The user manuals can be downloaded from *olink.com/downloads.*

3.2 Other resources

The following resources are available from the Olink website for technology information, protocol guidance and lists of available biomarker assays.

Guidelines

- 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*

White papers

- PEA - a high multiplex immunoassay technology with qPCR or NGS readout
  - Describes the PEA technology including the internal and external controls, QC and its advantages for medium-to-large-scale protein biomarker studies.
- Pre-analytical variation in protein biomarker research
  - Provides guidance on factors to consider when collecting, storing and handling samples for use in protein biomarker studies.
- Strategies for design of protein biomarker studies
  - Describes important aspects of study design to consider, such as adequate sample size, control of confounding factors and biases, and appropriate statistical analysis.

The white papers can be downloaded from *olink.com/downloads.*

3.3 Videos

- The Promise of Proteomics
  - Introduction to Olink Explore and how it can help to accelerate proteomics research. *olink.com/our-platform/promise-of-proteomics/*
- PEA technology animation video
  - Explains the principles of PEA with NGS and qPCR readout. */olink.com/our-platform/our-pea-technology/.*

4. Technical support

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

5. Safety

5.1 Safety instructions

Follow general laboratory safety procedures:

- Use gloves, safety goggles and protective clothing when performing the experiments.
- Handle and dispose of hazardous sample material according to local regulations.

5.2 Hazardous chemicals

See safety data sheet (SDS), available from the Olink website: *olink.com/downloads*.

6. Technology description

6.1 About PEA™

The technology behind the Olink panels is called Proximity Extension Assay (PEA). The Olink Explore HT platform (PEA with NGS readout) is comprised of a sample preparation that generates an Olink library per block, 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):

Day 1:

1. Sample preparation and dilution

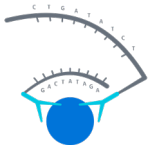
Duration of instrument protocols: 36 min

Samples and controls are transferred to one sample source plate and samples are diluted.

2. Immuno reaction (Incubation)

Duration of instrument protocols: 50 min
Duration of incubation: 16-24h (performed overnight)

High multiplexed PEA™ probes, matched pairs of antibodies with unique DNA oligonucleotides, bind to their respective proteins in the samples.

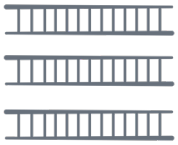


Day 2:

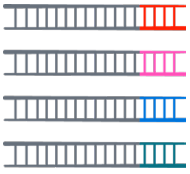
3. PCR

Duration of PCR program: 2 h

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). Unique sample indexes are added to every sample, to allow pooling of the DNA amplicons for all samples.



The final DNA amplicons in the Olink Explore HT libraries include:



- Specific barcode sequences for each assay

- Sample specific indexes

- Required sequences for Illumina sequencing (P5 and P7 Adapters and Sequencing Primer Binding Site Rd1SP)

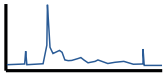
4. PCR pooling

Duration of instrument protocols: 40 min

Samples from the same dilution block are pooled together, resulting in one pool per dilution block, each containing 192 samples/controls.

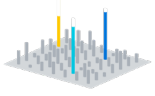
5. Library purification and quality control

Each pool is purified using magnetic beads. The quality of each Olink Explore HT library is assessed through automated electrophoresis.



6. Sequencing

The Olink Explore HT library is sequenced by NGS using Illumina® platform. The relative concentration of each biomarker, based on matched counts (the number of reads for each specific combination of sample and assay), is calculated using the NPX™ Explore HT & 3072 or Olink Explore CLI software.

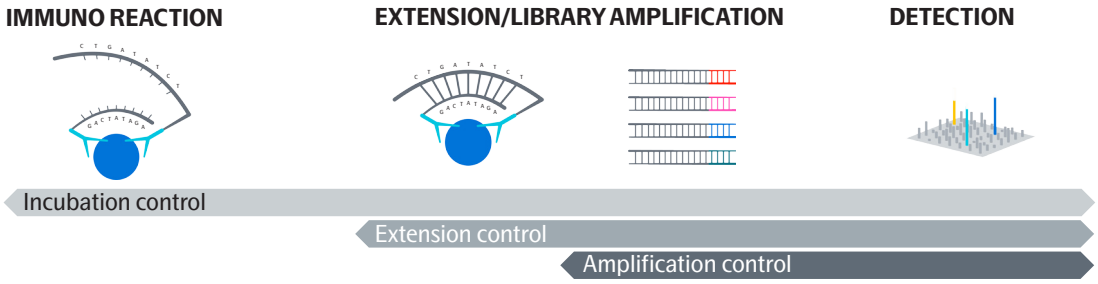


6.2 Quality control

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

6.2.1 Internal controls

Three internal controls are spiked into every sample for each block. 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.

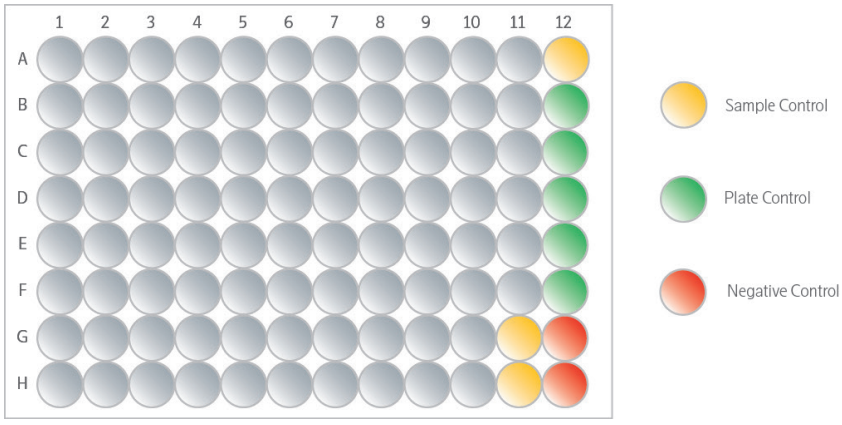
Extension Control: The Extension Control is composed of an antibody coupled to a unique pair of DNA-tags. These DNA-tags are always in proximity, so that this control is expected to give a constant signal independently of the immuno reaction. This control monitors variation in the extension and amplification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

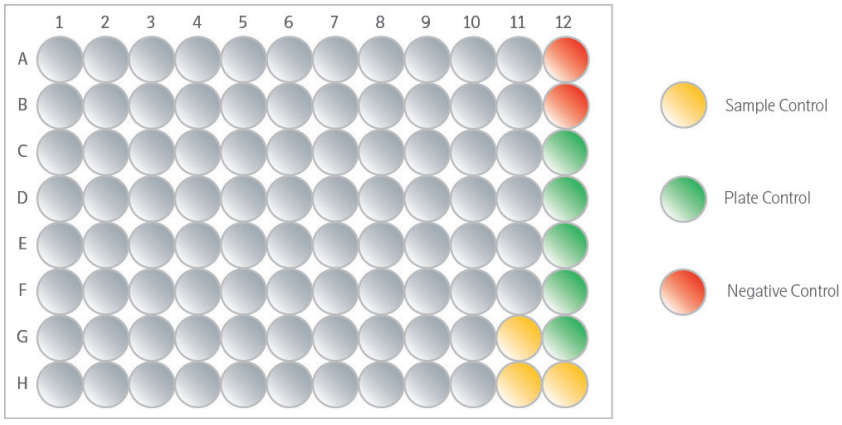
Amplification Control: The Amplification Control is a complete double stranded DNA amplicon which does not require any proximity binding or extension step to generate a signal. This control monitors the amplification/sample indexing step.

6.2.2 External controls

External controls are separate samples that are used for different purposes. There are seven required and three recommended external controls that are added to separate wells on each of the two sample plates. The figures below shows the two sample plate layouts, with 86 samples, 2 Negative Controls, 3 Sample Controls (optional), and 5 Plate Controls (required).

IMPORTANT: *If using other sample matrices than plasma or serum, please contact support@olink.com before proceeding with the sample preparation as the positions of the external controls in the Sample Source Plate differ.*





Sample Control: Sample Control is included in triplicates 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.

Plate Control: Plate Control is included in 5 replicates on each plate. The median of the Plate Control replicates is used to normalize each assay and to compensate for potential variation between runs and plates.

Negative Control: Negative Control is included in duplicate 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 assess potential contamination of assays.

7.Guidelines

7.1 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.

7.2 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.*

7.3 Pipetting techniques

Both forward and reverse pipetting are used in the Olink Explore HT 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.

7.3.1 General pipetting guidelines

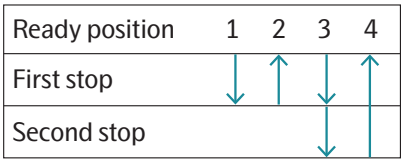
- Calibrate all pipettes regularly (at least with a 6-month interval).
- Let the reagents and liquids reach room temperature before use to maximize accuracy.
- Pipette near the liquid surface.
- Do not turn the pipette on the side when there is liquid in the tip, as liquid might contaminate the interior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

7.3.2 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.



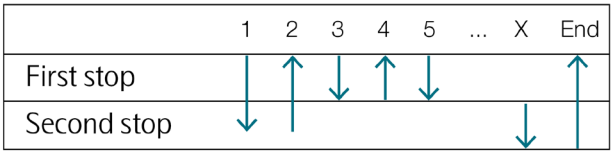
7.3.3 Reverse pipetting

Reverse pipetting improves precision with smaller volumes and viscous solutions, and is performed as follows:

1. Before filling the pipette with liquid, push the operating button past the first stop and then dip the tip into the liquid, just below the liquid surface.
2. Slowly release the operating button all the way up. This will fill the pipette tip with a volume larger than the set volume.

NOTE: *Ensure that all tips contain the exact same volume if a multichannel pipette is used.*

3. Press the operating button to the first stop to dispense the liquid into the recieving vessel. This volume is equal to the set volume, and a small volume will remain in the pipette tip. Hold the button pressed down to the first stop if the same pipette tip is going to be used to transfer the same liquid to another well.
4. Dip the pipette tip in the liquid again, and slowly release the operator button all the way up.
5. Continue pipetting into the recieving vessel.
   Repeat steps 4 and 5 as shown in the illustration, until liquid has been transferred into all wells.
6. Discard the small volume left in the pipette tip once the pipetting of the liquid is done. Release the operating button all the way up and discard the empty pipette tip.



7.4 Vortexing

Vortexing is performed using the MixMate, with settings according to:

Plate type	No adaptor	Tube Holder PCR 96	Tube Holder 25/50
96-wells, semi-skirted	–	2 000 rpm,30 sec	–
96-wells, skirted	2 500 rpm, 30 sec	–	–
384-wells	3 000 rpm, 30 sec	–	–
8-well strip	–	2 000 rpm,30 sec	–
50 mL tube	–	–	1 000 rpm, 30 sec

7.4.1 96-well semi-skirted plates vortexing

1. Insert the Tube Holder PCR 96 in the plate holder by holding it up against the back edge of the plate holder so that the stop pins fit in the holes.
2. Press the plate into the bores of the holder and make sure they sit evenly. Note that misplacement of the plate will result in uneven mixing of the wells and low-quality data.
3. Set mixing speed and time according to the table above.
4. Start mixing by pressing the start/stop key. After 30 seconds, MixMate will automatically stop.
5. Take the plate out of the holder.
6. Remove the holder by lightly pressing the Push release button.

7.4.2 384- and 96-well skirted Plates vortexing

1. Insert the appropriate 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 the table above.
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.

7.4.3 8-well strip vortexing

1. Insert the Tube Holder PCR 96 in the plate holder by holding it up against the back edge of the plate holder so that the stop pins fit in the holes.
2. Push the 8-well strip fully into the bores of the holder. Make sure that the strip sits tight and flat. Note that uneven placement of the strip will result in uneven mixing and low quality-data.
3. Set mixing speed and time according to the table above.
4. Start mixing by pressing the start/stop key. After 30 seconds, MixMate will automatically stop.
5. Gently take the 8-well strip out of the holder. The rubber on top of the holder is sticky, so make sure that the lids do not open while removing the strip.
6. Remove the holder by lightly pressing the Push release button.

7.4.4 50 mL tube vortexing

1. Insert the Tube Holder 25/50 in the plate holder by holding it up against the back edge of the plate holder so that the stop pins fit in the holes.
2. Push the 50 mL tube fully into one of the bores of the holder.
3. Set mixing speed and time according to the table above.
4. Start mixing by pressing the start/stop key. After 30 seconds, MixMate will automatically stop.
5. Take the 50 mL tube out of the holder.
6. Remove the holder by lightly pressing the Push release button.

8.Required equipment and consumables

This chapter lists everything that is required to perform an experiment using the Olink Explore HT protein biomarker platform, excluding the Olink Explore HT 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 HT Reagent Kits, refer to *2. Olink® Explore HT Reagent Kit contents.*

8.1 Important information

The Olink Explore HT 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.

8.2 Olink softwares

8.2.1 NPX™ Explore HT & 3072 software

NPX Explore HT & 3072 is an analysis software specifically designed for the Olink Explore HT analysis platform. It comes with an accompanying pre-processing software and is required for the generation and analysis of counts files for completed Olink Explore HT runs.

For further information, refer to the NPX™ Explore HT & 3072 User Manual.

8.2.2 NPX™ Explore CLI HT & 3072 software

NPX Explore CLI HT & 3072 is a command-line interface (cli) for the Olink Explore HT product. The application is capable of performing normalization, quality control (QC) and CV computations on NGS data and exporting the results on several supported formats.

8.3 Instruments

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

Instrument specifications

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®	813647B
	F.A.S.T.™ Adapter block for semi-skirted and non skirted PCR plates.	Pre-PCR/ Post-PCR	Formulatrix®	816409B
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
	epMotion® TS 50 single-channel dispensing tool, 1–50 uL (x 2) (Optional, needed if running fully automated PCR pooling)	Post-PCR	Eppendorf®	5280000010 NA: 960001010
	Reservoir rack	Post-PCR	Eppendorf®	5075754002 NA: 9600002148
	epMotion® Rack for 24 tubes, 1.5/2.0 mL (Optional, needed if running fully automated PCR pooling)	Post-PCR	Eppendorf®	5075751275
	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 1000 µL 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
	Tall Tip Pickup Adapter MPH96	Post-PCR	Hamilton	6608647-01
	3 SMP-CAR-32, Set of 3 carriers	Post-PCR	Hamilton	173410
	Tube Carrier Insert Eppendorf 1.5mL, Set of 3	Post-PCR	Hamilton	182238
E	**MixMate®**	**Pre- and Post-PCR**	**Eppendorf®**	**5353000510 (230V Version)** **5353000529 (110V Version)**
	Tube Holder PCR 96		Eppendorf®	5353040113
	Tube Holder 25/50 mL		Eppendorf®	5353040156
F	**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**	**Post-PCR**	**Illumina**	**20012850**
G2	**NovaSeq™ X Plus**	**Post-PCR**	**Illumina**	**20084804**

8.4 Equipment and consumables: Pre-PCR

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

8.4.1 Sample dilution, Incubation and PCR

Equipment

Equipment	Supplier	Article number
F.A.S.T.™ instrument including accessories	For specifications, refer to *8.3 Instruments*	
Mosquito® LV including accessories	For specifications, refer to *8.3 Instruments*	
Dragonfly® discovery 3 head including accessories	For specifications, refer to *8.3 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, 5–50 µL (Optional: adjustable)	Any	–
Plate centrifuge	Any	–
MixMate® controlled plate vortex with holders	Eppendorf®	5353000510 NA: 5353000529
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	–

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 NA: 951020702
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)***	Formulatrix®	233590
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.

8.5 Equipment and consumables: Post-PCR

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

8.5.1 Pooling of PCR products

Equipment

Equipment	Supplier	Article number
2 x ProFlex™ 2 x 384-well PCR System	For specifications, refer to *8.3 Instruments*	
Formulatrix F.A.S.T.™ including accessories*	For specifications, refer to *8.3 Instruments*	
epMotion® 5075l including accessories*	For specifications, refer to *8.3 Instruments*	
Hamilton Microlab STAR® including accessories*	For specifications, refer to *8.3 Instruments*	
Manual pipettes: 10–100 µL (Optional)	Any	–
MixMate® controlled plate vortex	Eppendorf®	5353000510
Plate centrifuge	Any	–
Plate vortex	Any	–
Microcentrifuge with inserts for both tubes and 8-strip (high speed not necessary)	Any	–

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

Consumables

Consumables	Supplier	Article number
MicroAmp™ Clear Adhesive Film	Thermo Fisher Scientific	4306311 /100x
Plate sealer	Any	–
Filter pipette tips (compatible with manual pipettes) (Optional)	Any	–
Microcentrifuge tubes, 1.5 mL	Any	–

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 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)
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

8.5.2 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	–

8.5.3 Quality control of Olink® libraries

Equipment

Equipment	Supplier	Article number
2100 Bioanalyzer System including accessories	For specifications, refer to *8.3 Instruments*	
4200 TapeStation System including accessories*	For specifications, refer to *8.3 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

Consumables	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

8.5.4 Next generation sequencing using NovaSeq™ 6000

Equipment

Equipment	Supplier	Article number
NovaSeq™ 6000 including accessories	For specifications, refer to *8.3 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
S4	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	–

For assistance, consult a statistician or contact Olink Support before running the study.

- The 3 replicates of sample control are optional.
- Consult the Olink white paper "Strategies for design of protein biomarker studies" and the sample randomization guidelines. For links, refer to *3. Associated documentation and resources.*

NOTE: *Sample randomization helps to ensure that technical variation does not overlap with biological variation.*

8.5.5 Next generation sequencing using NovaSeq™ X Plus

Equipment

Equipment	Supplier	Article number
NovaSeq™ X Plus including accessories	For specifications, refer to *8.3 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

Flow cell specific consumables	Supplier	Article number
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	–

9.Revision history

Version	Date	Description
4.0	2024-10-07	*2.2* Index Plate A and B replaced by Index Plate C. Part 1 and Part 2 removed from this manual. Name change of manual to Olink® Explore HT Overview User Manual. Editorial changes.
3.0	2024-06-19	Prepare PCR Plates and perform PCR: step 9: spin down added. Laboratory instructions for 86 samples, added.
2.1	2024-04-23	*8.3* Precision Tab Carrier, MTP, L5 added back to D2 in table.
2.0	2024-04-16	*3.1* updated. *8.2* updated. *8.3* Article number for MixMate Tube Holder PCR 96 changed. Precision Tab Carrier for Hamilton STAR deleted. *8.3*, *8.4.1*, and *8.5.1*: F.A.S.T. added as post-PCR instrument. *8.3*, *8.5.3*, and Quality control: TapeStation added. *8.5.5* added. Important information: Vortex table added. Pool PCR products: F.A.S.T. added as post-PCR instrument. Perform Sample Dilution: Information added to Important: "Make sure to use the correct Mixmate settings as incorrect settings may lead to low-quality data". Next Generation Sequencing: Illumina® NovaSeq X Plus added. Safe stopping point adding at the end of the Pool PCR Products sections. Name change of Olink® NPX Explore HT to NPX™ Explore HT & 3072.
1.3	2024-01-26	*6.2.2* and Prepare the samples: Important regarding Plate layout for alternative plate matrices added. Perform Sample Dilution for Mosquito, bullet 5: Information added to Important: "Make sure to use the correct Mixmate settings as incorrect settings may lead to low-quality data". Prepare Reagent Source Plate: Instructions bullet 2: reverse pipetting added. Index dispensation, bullet 4: changed to "in the magnetic booster plates" and bullet 2: rpm for MixMate changed to 3000. Perform Sample Dilution for F.A.S.T.: duration time changed to 3 hours.
1.2	2023-10-05	*2.2* Olink Explore HT Sample Prep article number updated. PCR Enzyme and Index Plate Cap colors and article number updated and note added. *8.3* A1 - both article numbers updated. D2 - Tall tip Pickup adapter, name and article number updated. Prepare the samples: well numbers in Sample Plate 2 updated. Perform sample dilution: Volume of transferred diluted 1:1000 samples corrected (0.3 µL) Incubation for Mosquito: Illustration for Instructions Part 3 corrected (left position empty). 13 and 27: unit for milliQ water corrected (mL). Index and PCR setup: updated for 384-well Index Plates.
1.1	2023-09-15	Part 4: Rerun removed.
1.0	2023-08-08	New

www.olink.com

1293, v4.0, 2024-10-07