Olink® Explore Post-PCR using Hamilton STAR®

User Manual

1310, v1.0, 2022-12-21

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1. Introduction

1.1 Intended use

Olink® Explore is a multiplex immunoassay platform for human protein biomarker discovery. The product is intended for Research Use Only, and not for use in diagnostic procedures. The laboratory work shall only be run by trained laboratory staff. Data processing shall only be performed by trained staff. The results are meant to be used by researchers in conjunction with other clinical or laboratory findings.

1.2 About this manual

This manual is a complement to the Olink Explore User Manuals and provides the additional instructions needed to run the post-PCR workflow using Hamilton STAR. This manual needs to be used together with the applicable user manual (refer to *1.4 Associated Documentation)* for the following Olink Explore Reagent Kits:

- Olink® Explore 384 Reagent Kit
- Olink® Explore 4x384 Reagent Kit
- Olink® Explore 1536 and Expansion Reagent Kit
- Olink® Explore 3072 Reagent Kit
- Olink® Explore 3072 Reagent Kit (384 samples)

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

Prior to starting the laboratory workflow, consult the Olink® Explore Overview User Manual for an introduction to the Explore platform, including information about equipment and documentation needed, an overview of the workflow, as wells as laboratory guidelines. Refer to the laboratory instructions for the applicable Olink Explore User Manual for all steps prior to 2.7 Pool PCR1 products and all steps after 2.9 Pool PCR2 products.

1.3 Reagents included

For information regarding reagents, refer to the applicable Olink Explore User Manual, refer to *1.4 Associated Documentation.*

1.4 Associated Documentation

- Olink® Explore Overview User Manual, doc nr 1187
- Olink® Explore 384 User Manual, doc nr 1188
- Olink® Explore 4 x 384 User Manual, doc nr 1189
- Olink® Explore 1536 & Expansion User Manual, doc nr 1190
- Olink® Explore 3072 User Manual, doc nr 1191

All relevant Olink documentation is available from the Olink website: https://www.olink.com/downloads.

1.5 Technical support

For questions, guidance and support, contact Olink Proteomics at support@olink.com.

2. Laboratory instruction for Post-PCR using Hamilton STAR®

This section provides instructions on how to perform each Post-PCR step of the Olink® Explore laboratory workflow using Hamilton STAR®. These steps replace the following steps in the workflow manuals:

- Pool PCR1 products
- Amplification and sample indexing (PCR2)
- Pool PCR2 products

2.1 Pool PCR1 products using Hamilton STAR®

During this step, the PCR1 products from the PCR1 Plates are pooled into one PCR1 Pooling Plate using the Hamilton STAR instrument (*Figure 1*).

Prepare bench

- PCR1 Plates, prepared in previous step
- MilliQ water (at +4 °C, preferably kept in the fridge until use)
- 1x 384-well PCR plate (skirted)
- 1x water reservoir (300 mL)
- 50 µL Conductive Filter Tips (1x rack per panel, 1x rack for water)
- Adhesive films
- Temperature-resistant labels or marker pen

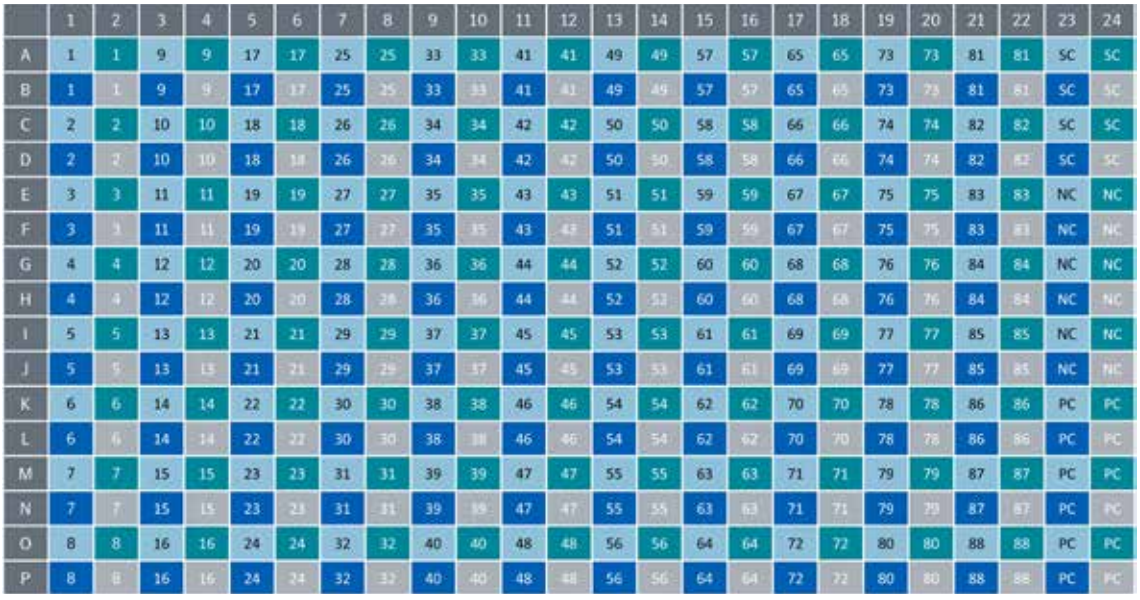
Before you start

- Thaw PCR1 Plates at room temperature if frozen.
- Mark the new 384-well PCR plate: "PCR1 Pooling Plate".
- Switch on the Hamilton STAR system, and open the Hamilton Run Control software.

Instructions

1. Make sure that PCR1 Plates are thawed and properly sealed, then vortex the plates and spin down at 400–1000 x g for 1 minute at room temperature.
2. Inspect the wells of PCR1 Plates to make sure that no liquid has evaporated and that all the liquid is at the bottom of the wells. Spin down the plates again if necessary.
3. In Run Control, select the protocol *PCR1 Pooling* and click the **Start** button.
4. Select the number of Destination Pooling Plates and the number of Panels being Pooled for each, then click **OK**.
5. Pull out all carriers to the load position and prepare the Hamilton STAR deck according to the software instructions.
   - Add MilliQ water to the reservoir.
   - Place PCR1 Plates on the carrier.
   - Carefully remove the adhesive films.
   - Fill Tip carrier in selected positions.
   - Push in Water Reservoir manually.

6. Click **OK** in the software to load the tip and plate carriers automatically and begin the run.
   Result: The Hamilton STAR automatically scans the tips, dispenses 12 µL MilliQ water into each well of the PCR1 Pooling Plate, and pools 3 µL of each PCR1 product from each sample into one well per panel. The run takes approximately 6 minutes to be completed.
7. When the protocol is finished, remove the PCR1 Pooling Plate and seal it with a new adhesive film. Keep the Hamilton STAR on for later use.
8. Vortex the PCR1 Pooling Plate and spin down at 400–1000 x g for 1 minute.
9. Inspect the PCR1 Pooling Plate to ensure that all wells contain the same amount of liquid (24 µL).
10. Remove PCR1 Plates containing the remaining PCR products and seal them with new adhesive films. Store them at 20 °C for up to 2 weeks in case of potential reruns.
11. Continue to *2.2 Amplification and sample indexing (PCR2) using Hamilton STAR®*, or store the PCR1 Pooling Plate at +4 °C until ready to use (the same day).



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B	1	1	9	9	17	17	25	25	33	33	41	41	49	49	57	57	65	65	73	73	81	81	SC	SC
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D	2	2	10	10	18	18	26	26	34	34	42	42	50	50	58	58	66	66	74	74	82	82	SC	SC
E	3	3	11	11	19	19	27	27	35	35	43	43	51	51	59	59	67	67	75	75	83	83	NC	NC
F	3	3	11	11	19	19	27	27	35	35	43	43	51	51	59	59	67	67	75	75	83	83	NC	NC
G	4	4	12	12	20	20	28	28	36	36	44	44	52	52	60	60	68	68	76	76	84	84	NC	NC
H	4	4	12	12	20	20	28	28	36	36	44	44	52	52	60	60	68	68	76	76	84	84	NC	NC
I	5	5	13	13	21	21	29	29	37	37	45	45	53	53	61	61	69	69	77	77	85	85	NC	NC
J	5	5	13	13	21	21	29	29	37	37	45	45	53	53	61	61	69	69	77	77	85	85	NC	NC
K	6	6	14	14	22	22	30	30	38	38	46	46	54	54	62	62	70	70	78	78	86	86	PC	PC
L	6	6	14	14	22	22	30	30	38	38	46	46	54	54	62	62	70	70	78	78	86	86	PC	PC
M	7	7	15	15	23	23	31	31	39	39	47	47	55	55	63	63	71	71	79	79	87	87	PC	PC
N	7	7	15	15	23	23	31	31	39	39	47	47	55	55	63	63	71	71	79	79	87	87	PC	PC
O	8	8	16	16	24	24	32	32	40	40	48	48	56	56	64	64	72	72	80	80	88	88	PC	PC
P	8	8	16	16	24	24	32	32	40	40	48	48	56	56	64	64	72	72	80	80	88	88	PC	PC

Figure 1. PCR1 Pooling Plate layout.
The numbers indicate the sample numbers. The colors indicate the panel numbers: light blue = Panel 1, green = Panel 2, dark blue = Panel 3, grey = Panel 4.

2.2 Amplification and sample indexing (PCR2) using Hamilton STAR®

During this step, a PCR2 Mix is prepared manually and then mixed with the samples along with index primers (unique for each sample) using the Hamilton STAR. The samples are then subjected to a second PCR reaction.

2.2.1 Prepare PCR2 Mix

During this step, a PCR2 Mix is prepared manually. The PCR2 Mix must be used within 15 minutes from preparation.

Prepare bench

- PCR1 Pooling Plate, prepared in previous step
- Olink® Explore PCR2 Solution
- Olink® Explore PCR2 Enzyme (when brought to the lab bench, keep the PCR2 Enzyme in a freezing block (-20 °C))
- Olink® Explore Index Plate 1
- MilliQ water (at +4 °C, preferably kept in the fridge until use)
- Falcon tube (15 mL)

- Manual pipettes (10–100 µL, 100–1000 µL)
- Filter pipette tips
- Temperature-resistant labels or marker pen

Before you start

- Thaw the PCR2 Solution and the Index Plate 1 at room temperature.
- If stored at +4 °C, allow the PCR1 Pooling Plate to reach room temperature.
- Mark the new 15 mL tube: "PCR2 Mix".
- Switch on one ProFlex™ PCR instrument. No preheating is required.

Instructions

1. Vortex the PCR2 Solution and spin it briefly.
2. Vortex the Index Plate 1 and spin it at 400–1000 x g for 1 minute.
3. Make sure that the PCR1 Pooling Plate is properly sealed, then vortex it and spin at 400–1000 x g for 1 minute.
4. Spin the PCR2 Enzyme briefly. Do not vortex.

NOTE: *Always keep the PCR2 Enzyme at -20 °C. When brought to the lab bench, keep it in a freezing block (-20 °C).*

5. Prepare a PCR2 Mix in a Falcon tube following the order and volumes indicated in *Table 1*.

Table 1. PCR2 Mix

Addition order	Reagent	Volume (µL)
1	MilliQ water (+4 °C)	7 076
2	PCR2 Solution	1 015
3	PCR2 Enzyme	21
	Total	8 112

5. Vortex the PCR2 Mix thoroughly. Aliquot to 96 well PCR plate with at least 72 uL/well. Keep at room temperature until use.

TIME SENSITIVE STEP: *Start the dispensing of the PCR2 Mix within 15 minutes from preparation.*

2.2.2 Prepare PCR2 Plate and perform PCR2

During this step, the prepared PCR2 Mix is mixed with the samples along with index primers using the Hamilton STAR. The samples are then subjected to a second PCR reaction (*Figure 3*).

Prepare bench

- PCR1 Pooling Plate (at room temperature), prepared in previous step
- PCR2 Mix, prepared in previous step
- Olink® Explore Index Plate 1
- 1x 384-well PCR plate (skirted)
- 1x 96-well PCR plate with PCR2 Master Mix (skirted), prepared in previous step
- 50 µL Conductive Filter Tips (6x rack per PCR2 plate)
- Adhesive films
- Temperature-resistant labels or marker pen

Before you start

- Mark the new 384-well PCR plate: “PCR2 Plate”.

Instructions

1. Open Hamilton Run Control and select the protocol *PCR2_Setup* and click **Start.**
2. Select the number of PCR2 plates being prepped and the number of Panels in each plate, and click **OK**.
3. Centrifuge Index Plate 1 at 400–1000 x g for 1 minute and inspect the plate to ensure that all wells contain the same amount of liquid (20 µL). If a well is empty or contains a lower volume, contact Olink Support.
4. Carefully remove the adhesive films from the Index Plate 1 and the PCR1 Pooling Plate. Make sure that no air bubbles are trapped at the bottom of the wells of the PCR1 Pooling Plate.
5. Pull out all carriers and prepare the Hamilton STAR deck according to the software instructions.
6. Click **OK** once the deck is loaded, then the system will automatically load the carriers and begin the protocol.
   Result: Hamilton STAR transfers 16 µL of PCR2 Mix, 2 µL of Index Primers (from the Index Plate 1) and 2 µL of PCR1 products (from the PCR1 Pooling Plate) into the applicable wells of the PCR2 Plate (Figure 3). The run takes approximately 8 minutes to be completed.
7. When the protocol is finished (indicated by a message in the software), remove the PCR2 Plate and seal it with a new adhesive film. Keep the Hamilton STAR on for later use.

IMPORTANT: *All wells must be properly sealed to avoid evaporation of the samples.*

TIME SENSITIVE STEP: *Start the PCR2 program within 5 minutes from end of Hamilton STAR protocol.*

8. Vortex and spin down at 400–1000 x g for 1 minute at room temperature.
9. Inspect the PCR2 Plate to ensure that all wells contain the same amount of liquid (20 µL).
10. Place the PCR2 Plate in the ProFlex and add a balance plate to the other side of the ProFlex.
11. Click Open and select the program *Olink Index PCR2* (*Figure 2*). Click **Start**.

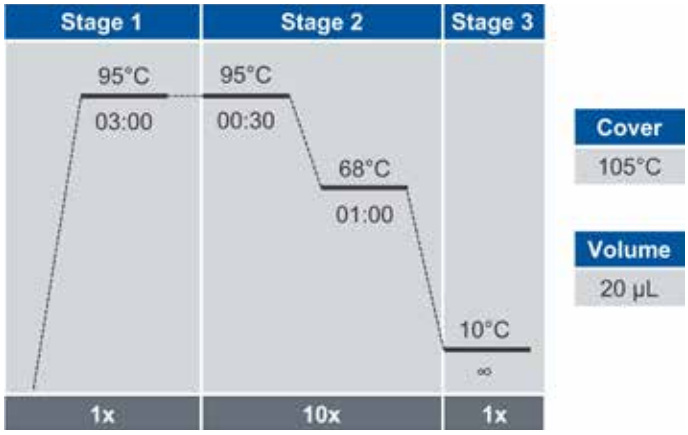

Figure 2. Olink Index PCR2 program

12. Remove the PCR1 Pooling Plate containing the remaining PCR products from the Hamilton STAR and seal it with a new adhesive film. Store it at -20 °C for up to 2 weeks in case of potential reruns.
13. Discard the Index Plate 1.
14. When the PCR program is finished (~25 minutes), continue to *2.3 Pool PCR2 products using Hamilton STAR®*, or store the PCR2 Plate at +4 °C until use (the same day).

SAFE STOPPING POINT: *The PCR2 Plate can be stored at -20 °C for up to 2 weeks.*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	1	9	9	17	17	25	25	33	33	41	41	49	49	57	57	65	65	73	73	81	81	SC	SC
B	1	1	9	9	17	17	25	25	33	33	41	41	49	49	57	57	65	65	73	73	81	81	SC	SC
C	2	2	10	10	18	18	26	26	34	34	42	42	50	50	58	58	66	66	74	74	82	82	SC	SC
D	2	2	10	10	18	18	26	26	34	34	42	42	50	50	58	58	66	66	74	74	82	82	SC	SC
E	3	3	11	11	19	19	27	27	35	35	43	43	51	51	59	59	67	67	75	75	83	83	NC	NC
F	3	3	11	11	19	19	27	27	35	35	43	43	51	51	59	59	67	67	75	75	83	83	NC	NC
G	4	4	12	12	20	20	28	28	36	36	44	44	52	52	60	60	68	68	76	76	84	84	NC	NC
H	4	4	12	12	20	20	28	28	36	36	44	44	52	52	60	60	68	68	76	76	84	84	NC	NC
I	5	5	13	13	21	21	29	29	37	37	45	45	53	53	61	61	69	69	77	77	85	85	NC	NC
J	5	5	13	13	21	21	29	29	37	37	45	45	53	53	61	61	69	69	77	77	85	85	NC	NC
K	6	6	14	14	22	22	30	30	38	38	46	46	54	54	62	62	70	70	78	78	86	86	PC	PC
L	6	6	14	14	22	22	30	30	38	38	46	46	54	54	62	62	70	70	78	78	86	86	PC	PC
M	7	7	15	15	23	23	31	31	39	39	47	47	55	55	63	63	71	71	79	79	87	87	PC	PC
N	7	7	15	15	23	23	31	31	39	39	47	47	55	55	63	63	71	71	79	79	87	87	PC	PC
O	8	8	16	16	24	24	32	32	40	40	48	48	56	56	64	64	72	72	80	80	88	88	PC	PC
P	8	8	16	16	24	24	32	32	40	40	48	48	56	56	64	64	72	72	80	80	88	88	PC	PC

Figure 3. PCR2 Plate layout
The numbers indicate the sample numbers. The colors indicate the panel numbers: light blue = Panel 1, green = Panel 2, dark blue = Panel 3, grey = Panel 4.

2.3 Pool PCR2 products using Hamilton STAR®

During this step, all PCR2 products belonging to the same panel are pooled into one column of the PCR2 Pooling Plate using the Hamilton STAR (*Figure 4*). The Olink libraries are then manually transferred to one microcentrifuge tube per panel. Each tube contains amplicons from 96 samples, including controls.

Prepare bench

- PCR2 Plate, prepared in previous step
- 1x 96-well PCR plate (skirted)
- Microcentrifuge tubes (1.5 mL, 1x tube per panel)
- 50 µL Conductive Filter Tips (1x rack per pooling plate)
- Manual pipette (10–100 µL)
- Filter pipette tips
- Adhesive films
- Temperature-resistant labels or marker pen

Before you start

- Thaw PCR2 Plate at room temperature if frozen.
- Mark the new 96-well PCR plate: ”PCR2 Pooling Plate”.
- Mark four new microcentrifuge tubes: ”PCR2 [1–4, depending on the number of panels]”.

Instructions

1. Open Hamilton Run Control and select the applicable protocol *PCR2_Final_Pooling* and click **Start**.
2. Select the number of Destination Pooling Plates and the number of Panels being pooled in each, then click **OK**.
3. Make sure that the PCR2 Plate is thawed and properly sealed, then vortex and spin it down at 400–1000 x g for 1 minute at room temperature to ensure that all wells are mixed.
4. Inspect the wells of the PCR2 Plate to make sure that no liquid has evaporated and that all the liquid is at the bottom of the wells.

5. Pull out all carriers an prepare the Hamilton STAR deck according to the software instructions.
6. Once loaded, click **OK** to automatically load the carriers and begin the protocol.
   Result: Hamilton STAR pools 3 µL from each row of a given Panel into a single well of the PCR2 Pooling Plate. The result is one column of pooled PCR2 products per panel in the PCR2 Pooling Plate. (Figure 4).
7. When finished, remove the PCR2 Pooling Plate from the worktable. Seal the plate with a new adhesive film, vortex the plate and spin down at 400–1000 x g for 1 minute.
8. Inspect the PCR2 Pooling Plate to ensure that every applicable well contains the same amount of liquid (36 µL in columns 1, 3, 5 and 7).
9. Clear the Hamilton STAR and shut it down.

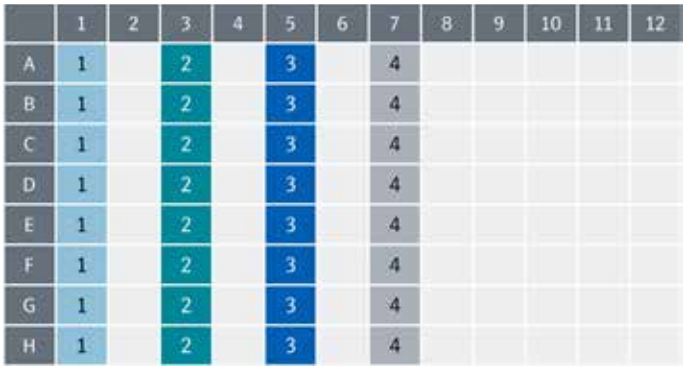

Figure 4. PCR2 Pooling Plate layout.
The numbers and colors indicate the panel numbers: light blue = Panel 1, green = Panel 2, dark blue = Panel 3, grey = Panel 4.

10. Carefully remove the adhesive film from the PCR2 Pooling Plate.
11. Using a single-channel pipette transfer the pooled PCR2 products from the PCR2 Pooling Plate to the PCR2 Tubes as described in *Table 2*. Use forward pipetting and change pipette tip after each well.

Table 2. Transfer PCR2 products. If running a single panel, only transfer from column 1 to PCR2 tube 1 will be applicable.

Volume (µL)	From column	To tube
30	1	PCR2 1
30	3	PCR2 2
30	5	PCR2 3
30	7	PCR2 4

12. Vortex the PCR2 Tubes and spin down briefly.
13. Remove the PCR2 Plate containing the remaining PCR2 products from the instrument, seal it with a new adhesive film and store it at -20 °C for up to 2 weeks in case of potential reruns.
14. Discard the PCR2 Pooling Plate.
15. Continue to the Library purification step in the workflow manual, or store the PCR2 Tubes at +4 °C until use (the same day).

SAFE STOPPING POINT: *The PCR2 Tubes can be stored at –20 °C for up to 2 weeks.*

3. Revision history

Version	Date	Description
1.0	2022-12-21	New

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1310, v1.0, 2022-12-21