

Olink® Flex and Olink® Target 48

Short instructions on how to run two kits on one Olink® 96.96 IFC

For each kit, first perform the Incubation and Extension steps and part 1 of the Detection step separately. The second part of the Detection step will be performed when the Sample Plates and Primer Plates from both kits are ready. If both kits are set up at the same time, they can share a 96-well plate.

NOTE: Only combine multiple kits for the same panel using the same panel data file on the same 96.96 IFC.

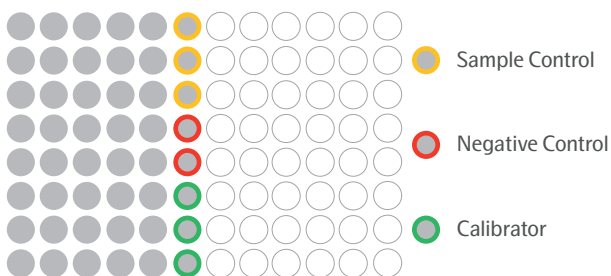
NOTE: Reagents from different kits should not be mixed or pooled, even if they are from the same lot.

Incubation

1. Prepare the Incubation mix in a microcentrifuge tube according to the table below.

| Incubation mix | per ½ 96-well plate (µL) |
|--------------------------------------|--------------------------|
| Olink® 1-48 plex Incubation Solution | 168 |
| Olink® Frw-probes | 21 |
| Olink® Rev-probes | 21 |
| Total | 210 |

2. Vortex and spin down the Incubation mix. Transfer 23 µL of the Incubation mix to each well of a new 8-well strip.
3. Transfer 3 µL of Incubation mix to each well of the first 6 columns of a 96-well plate by **reverse pipetting** and name the plate *Incubation Plate*.
4. Add 1 µL of each sample using a multi-channel pipette to the bottom of each well. To the control strip in column 6, add 1 µL of Sample Control to the three top wells (yellow), 1 µL of Negative Control to two wells (red), and 1 µL of Calibrators to three wells (green), according to the plate layout below.



5. Seal the plate with an adhesive plastic film, spin at 400 – 1000 x g, 1 min at room temperature. Incubate overnight at +4 °C.
6. Thaw the PEA Solution over night at +4 °C, and place the PEA Enhancer at room temperature over night.

Extension

1. Prepare an extension mix according to the table below.

| Extension mix | per ½ 96-well plate (µL) |
|-------------------------------|--------------------------|
| High Purity Water (+4 °C) | 4350 |
| Olink® 1-48 plex PEA Enhancer | 580 |
| Olink® 1-48 plex PEA Solution | 580 |
| Olink® 1-48 plex PEA Enzyme | 58 |
| Total | 5 568 |

2. Bring the *Incubation Plate* to room temperature, spin at 400 – 1000 x g for 1 min. Preheat the PCR machine.
3. Vortex the Extension mix and pour it into a multichannel pipette reservoir.
4. Start a timer for 5 min and transfer 96 µL of Extension mix to the upper part of the well walls of the *Incubation Plate* by using **reverse pipetting**.
5. Seal the plate with a new adhesive plastic film, use the MixMate® to vortex the plate at 2000 rpm for 30 sec, ensuring that all wells are mixed, and spin down.
6. Place the *Incubation Plate* in the thermal cycler and start the PEA program.
(50 °C 20 min, 95 °C 5 min (95 °C 30 sec, 54 °C 1 min, 60 °C 1 min) x 17, 10 °C hold)

Detection step: Part 1

1. Prepare a Detection mix according to the table below.

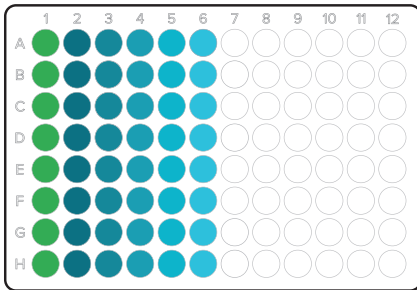
| Detection mix | per ½ 96-well plate (µL) |
|-------------------------------------|--------------------------|
| Olink® 1-48 plex Detection Solution | 275.0 |
| High Purity Water | 116.0 |
| Olink® 1-48 plex Detection Enzyme | 3.9 |
| Olink® 1-48 plex PCR Polymerase | 1.5 |
| Total | 396.4 |

2. Vortex the Detection mix and spin briefly and add 46 µL of the mix to each well of an 8-well strip.
3. Transfer 7.2 µL of the Detection mix to each well of column 1-6 in a new 96-well plate by **reverse pipetting**, and name it *Sample Plate*.
4. Remove the *Incubation Plate* from the thermal cycler, spin down the content and transfer 2.8 µL to the *Sample Plate*, using **forward pipetting**.
5. Seal the plate with an adhesive film, vortex and spin both at 400 – 1000 x g, 1 min at room temperature.

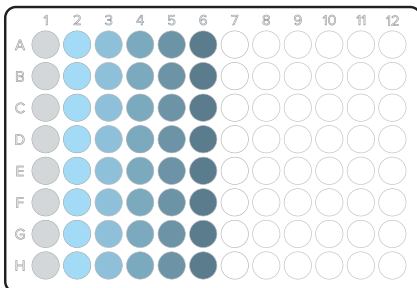
Detection step: Part 2

In this step, you will load the two Primer Plates and the two Sample Plates from kit 1 and 2 on the same Olink® 96.96 IFC.

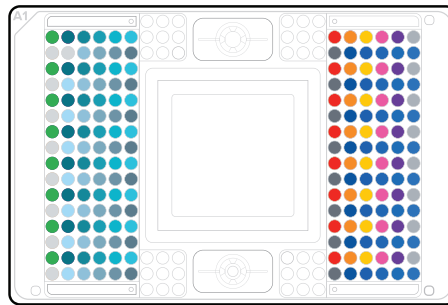
1. Thaw the *Primer Plates* from both kits, vortex and spin briefly.
2. Prepare and prime an Olink® 96.96 IFC for Protein Expression. Briefly, inject one Olink 96.96 IFC control line fluid syringe into each accumulator on the chip, remove the protective film from the bottom of the IFC and then prime the IFC on Olink® Signature Q100 following the instructions on the instrument screen.
3. Using a multichannel pipette, transfer 5 µL from each well of column 1-6 of the *Primer Plate* from kit 1 into the uneven rows of the primed 96.96 IFC left side inlets, and 5 µL from each well of column 1-6 of the *Primer Plate* from kit 2 into the even rows of the primed 96.96 IFC left side inlets. Use **reverse pipetting** and change tips after each primer. Do not leave any inlets empty.
4. Using a multichannel pipette, transfer 5 µL from each well of column 1-6 of the *Sample Plate* from kit 1 into the uneven rows of the primed 96.96 IFC right side inlets, and 5 µL from each well of column 1-6 of the *Sample Plate* from kit 2 into the even rows of the primed 96.96 IFC right side inlets. Use **reverse pipetting** and change tips after each sample. Do not leave any inlets empty.



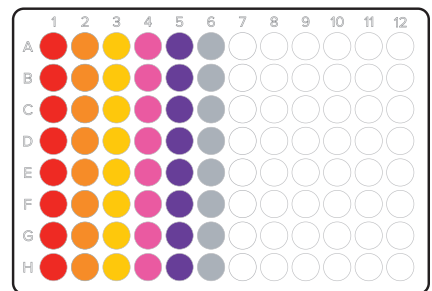
Primer Plate from kit 1



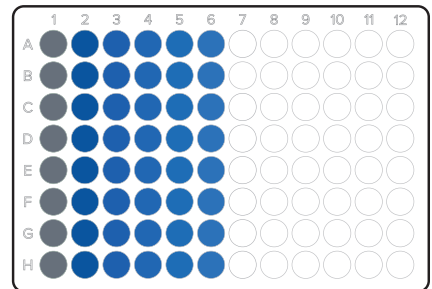
Primer Plate from kit 2



Option 1

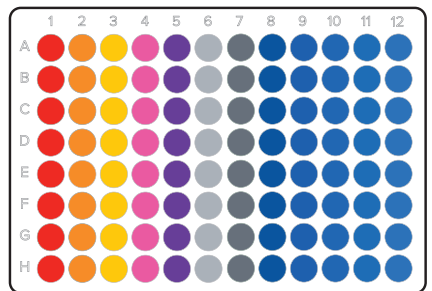


Sample Plate from kit 1



Sample Plate from kit 2

Option 2



Sample Plate for both kit 1 and kit 2

5. Remove any potential bubbles and load the IFC in Olink Signature Q100 and follow the instructions on the instrument screen.
6. Run the IFC on Olink Signature Q100.

Data analysis

This instruction applies to Olink® NPX Signature version 1.6.0.0 or later versions for Target 48, and an upcoming version for Olink Flex.

1. Under **Select Product**, choose your product and fill in the rest of the information about your project. Click **CREATE**.
2. Select your run(s).

NOTE: When Olink Flex or Target 48 have been run on Olink 96.96 IFCs, only runs made on 96.96 IFCs can be selected in this step. If other runs in the same project have been made on Olink® 48.48 IFCs, they can be uploaded later under **Study>Plates**. The same applies for runs on Olink 48.48 IFCs when previous runs have been made on Olink 96.96 IFCs.

3. The software will split your 96.96 IFCs into two separate plates, Plate 1 and Plate 2. In the **Annotate Run Data** screen annotate your run with the correct Panel data file.
4. Proceed with QC as usual.

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