Olink® Target 96

Short instructions

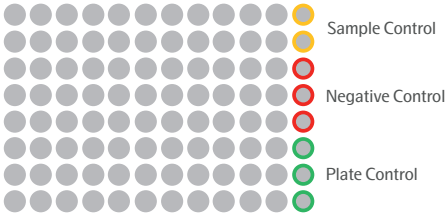
If running an Olink panel that requires pre-diluted samples, read section 7 Dilution step, in the Olink® Target 96 User Manual prior to starting.

Incubation

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

Incubation mix	per 96-well plate (µL)
Olink® Target 96 Incubation Solution	280
Olink® Target 96 Incubation Stabilizer	40
Olink® Target 96 A-probes	40
Olink® Target 96 B-probes	40
Total	**400**

2. Vortex and spin down the Incubation mix. Transfer 47 µL of the Incubation mix to each well of an 8-well strip.
3. Transfer 3 µL of Incubation mix to each well of a 96-well plate by **reverse pipetting** and name it *Incubation Plate*.
4. Add 1 µL of each sample to the *Incubation Plate,* using a multi-channel pipette, to the bottom of the well. In column 12, add 1 µL of Negative Control to three wells (red), and 1 µL of Interplate Control to three wells (green), according to the plate layout below. It is recommended to also run a pooled plasma sample as Sample Control (yellow) in two wells.



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.

Extension

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

Extension mix	per 96-well plate (µL)
High Purity Water	9385
Olink® Target 96 PEA Solution	1100
Olink® Target 96 PEA Enzyme	55
Olink® Target 96 PCR Polymerase	22
Total	**10 562**

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 into a multichannel pipette reservoir.
4. **Time sensitive step**
   Start a timer for 5 min and transfer 96 µL of Extension mix to the upper parts of the well walls of the Incubation Plate, by using **reverse pipetting**.

5. Seal the plate with an adhesive plastic film, use MixMate® to vortex thoroughly at 2500 rpm for 30 sec, ensuring that all wells are mixed, and spin down at 400 – 1000 x g for 1 min.
6. Place the *Incubation Plate* in the thermal cycler, and start the PEA program.
   (50 °C 20 min, 95 °C 5 min (95 °C 30s, 54 °C 1 min, 60 °C 1 min) x 17, 10 °C hold)

Detection

1. Prepare and prime an Olink® 96.96 IFC for Protein Expression. Briefly, inject one control line fluid syringe into each accumulator on the chip, and then prime the IFC in the Olink Signature Q100 instrument.
2. Thaw the *Primer Plate*, vortex and spin briefly.
3. Prepare a Detection mix according to the table below.

Detection mix	per 96-well plate (µL)
Olink® Target 96 Detection Solution	550
High Purity Water	230
Olink® Target 96 Detection Enzyme	7.8
Olink® Target 96 PCR Polymerase	3.1
Total	**790.9**

4. Vortex the Detection mix, spin briefly and add 95 µL to each well of an 8-well strip.
5. Transfer 7.2 µL of the Detection mix to each well of a new 96-well plate by **reverse pipetting** and name it *Sample Plate*.
6. Remove the *Incubation Plate* from the thermal cycler, spin down the content and transfer 2.8 µL from each well to the corresponding well on the *Sample Plate*, using **forward pipetting**.
7. Seal the plate with an adhesive plastic film, vortex and spin at 400 – 1000 x g, 1 min at room temperature.
8. Transfer 5 µL from each well of the *Primer Plate* and 5 µL of the *Sample Plate* into the primed 96.96 IFC left and right inlets, respectively. Use **reverse pipetting** and change tips after each primer or sample. Do not leave any inlets empty.
9. Remove bubbles and load the chip in the Olink Signature Q100 and follow the instructions on the instrument screen.
10. Run the plate on the Olink Signature Q100 and make sure that the correct interface plate is used.

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Olink Proteomics, Dag Hammarskjölds väg 52B , SE-752 37 Uppsala, Sweden

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