Olink® Target 48

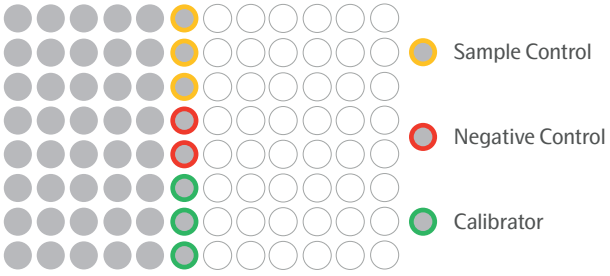
Short instructions

Incubation

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

Incubation mix	per ½ 96-well plate (µL)
Olink® Target 48 Incubation Solution	168
Olink® Target 48 Frw-probes	21
Olink® Target 48 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 the well, 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.



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 ½ 96-well plate (µL)
High Purity Water (+4 °C)	4350
Olink® Target 48 PEA Enhancer	580
Olink® Target 48 PEA Solution	580
Olink® Target 48 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 parts 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 2500 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

1. Prepare and prime an Olink® 48.48 IFC for Protein Expression. Briely, inject one control line fluid syringe into each accumulator on the chip, and then prime the IFC on the Olink® Signature Q100 following the instructions on the instrument screen.
2. Thaw the *Primer Plate*, vortex and spin briefly.
3. Prepare a Detection mix according to the table below.

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

4. Vortex the Detection mix and spin briefly and add 46 µL of the mix to each well of an 8-well strip.
5. 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*.
6. Remove the *Incubation Plate* from the thermal cycler, spin down the content and transfer 2.8 µL to the *Sample Plate*, using **forward pipetting**.
7. Seal the plate with an adhesive film, vortex and spin both at 400 – 1000 x g, 1 min at room temperature.
8. Transfer 5 µL from each well of the *Prime Plate* and 5 µL of the *Sample Plate* into the primed 48.48 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 IFC on the Olink Signature Q100.
11. Carefully remove the adhesive film from the *Primer Plate* to avoid contamination between wells.
12. Transfer 5 µL of each primer using **reverse pipetting** from each well in position 1 A-H (green) to the inlets in the first column on the left side of the IFC (green). Change pipette tips after each column. When using an eight-channel pipette every other inlet will be filled according to the image.

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