Olink® Flex

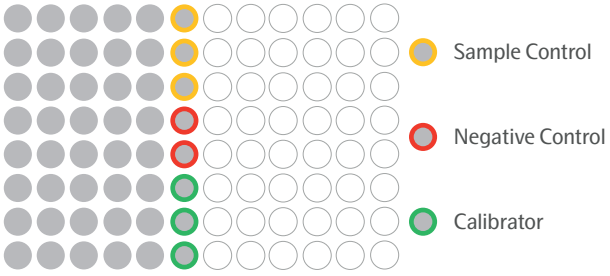
Short instructions

Incubation

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

Incubation mix	per ½ 96-well plate (µL)
Olink® 1-48 plex Incubation Solution	168
Olink® Flex Frw-probes	21
Olink® Flex 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.
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 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 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

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, 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.
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® 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**

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 column 1-6 of the *Primer Plate* and 5 µL from each well of column 1-6 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 IFC in Olink Signature Q100 and follow the instructions on the instrument screen.
10. Run the IFC on Olink Signature Q100.

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