Olink® Target 96

Short instructions

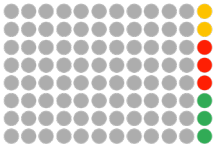
If running an Olink assay that requires pre-diluted samples, read the instructions in the Sample Dilution Guidelines prior to starting.

Incubation

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

Incubation mix	per 96-well plate (µL)
Incubation Solution	280.0
Incubation Stabilizer	40.0
A-probes	40.0
B-probes	40.0
Total	**400.0**

2. Vortex and spin down the Incubation mix. Transfer 47 µL of the Incubation mix to each well of an 8-well strip, by using reverse pipetting.
3. Transfer 3 µL of Incubation mix to a 96-well plate by reverse pipetting and name it *Incubation Plate*.
4. Add 1 µL of each sample using a multi-channel pipette to the bottom of the well, 1 µL of Negative Control to three wells (red), and 1 µL of Interplate Control to three wells (green), according to the plate layout. 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 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
PEA Solution	1100
PEA Enzyme	55
PCR Polymerase	22
Total	**10 562**

2. Bring the *Incubation Plate* to room temperature, spin at 400 x g for 1 min. Preheat the PCR machine.
3. Vortex the Extension mix and pour into a multi-channel 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 an adhesive plastic film, vortex thoroughly 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 30s, 54°C 1 min, 60°C 1 min) x17, 10°C hold).

Detection

1. Prepare and prime a 96.96 Dynamic Array™ Integrated Fluidic Circuit (IFC) according to the manufacturer's instructions. Briefly, inject one control line fluid syringe into each accumulator on the chip, and then prime the chip on the IFC Controller for approximately 20 minutes.
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)
Detection Solution	550.0
High Purity Water	230.0
Detection Enzyme	7.8
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 to the *Sample Plate*, using forward pipetting.
7. Seal the plate with an adhesive plastic film, vortex and spin at 400 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 Dynamic Array 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 Fluidigm IFC Controller HX according to the manufacturer's instructions.
10. Run the Olink Protein Expression 96×96 Program (50°C 120 s, 70°C 1800 s, 25°C 600 s, 95°C 300 s (95°C 15 s, 60°C 60 s) x 35) with the following settings: application-Gene Expression; passive Reference-ROX; assay-Single probe; probes-FAM- MGB) in the Fluidigm Biomark™ Reader according to the manufacturer's instructions.

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