SHORT INSTRUCTIONS

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

1. INCUBATION

- 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**

- Vortex and spin down the Incubation mix. Transfer 47 µL of the Incubation mix to each well of an 8-well strip using reverse pipetting.
- Transfer 3 µL of Incubation mix to a 96-well plate by reverse pipetting and name it *Incubation Plate*.
- 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, and 1 µL of Interplate Control to three wells, according to the plate layout.
- Seal the plate with an adhesive plastic film, spin at 400 x g, 1 min at room temperature. Incubate overnight at +4°C.

2. EXTENSION

- 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**

- Bring the *Incubation Plate* to room temperature, spin at 400 x g for 1 min. Preheat the PCR machine.
- Vortex the Extension mix and pour into a multi-channel pipette reservoir.

- 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.
- Seal the plate with an adhesive plastic film, vortex thoroughly ensuring that all wells are mixed, and spin down.
- 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).

3. DETECTION

- Prepare and prime a 96.96 Dynamic Array™ Integrated Fluidic Circuit (IFC) according to the manufacturer's instructions.
- Thaw the Primer Plate, vortex and spin briefly.
- 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**

- Vortex the Detection mix, spin briefly and add 95 µL to each well of an 8-well strip.
- 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*.
- Remove the *Incubation Plate* from the thermal cycler, spin down the content and transfer 2.8 µL to the *Sample Plate*.
- Seal the plate with an adhesive plastic film, vortex and spin at 400 x g, 1 min at room temperature.
- 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.
- Remove bubbles and load the chip in the Fluidigm IFC Controller HX according to the manufacturer's instructions.
- 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) x40) in the Fluidigm Biomark™ Reader according to the manufacturer's instructions.

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