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Sinai SCENT TMC - Olink Assay using Proseek® Multiplex 96×96 Kit (Juno Biomark System)

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We use this protocol and it's working

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Abstract

To describe the measurement procedure of 92 protein biomarkers in 1 µL sample volume through Proseek® Multiplex 96×96 Olink. The Proseek reagents are based on PEA, a Proximity Extension Assay technology, in which 96 oligonucleotide-labeled antibody pairs are allowed to bind to their respective protein targets in the sample. A PCR reporter sequence is formed by a proximity-dependent DNA polymerization event and is subsequently detected and quantified using real-time PCR. The assay is performed in a homogeneous 96-well format with no need for washing steps.

Guidelines

Note: Every panel has different Sample Preparation requirement for different sample matrices. Refer to the Step 1. For further details refer to the kit and biological sample specifics before commencing the assay.

Materials

- Proseek multiplex probe kit 96×96 (store at 4 °C)
- Proseek multiplex detection kit 96×96 (store at -20 °C)
- Proseek multiplex controls (store at -20 °C)
- Pipettes and Pipette filter tips
- 96 Well Multiply-PCR Plate half skirt
- Microcentrifuge tubes and FalconTM 15mL Conical Centrifuge Tubes
- 8-well strips tube with lids
- Multi-channel pipette reservoir
- Adhesive plastic film (heat-resistant)
- High purity water (sterile filtered, MilliQ or similar)
- Microcentrifuge for tubes and Centrifuge for plates
- Vortex
- VWR Reagent reservior 25ml
- Freezing block (-20 °C) for enzyme handling
- Refrigerator or cold room (2 °C to 8 °C)
- Bio-RAD T100 Thermal Cycle
- Fluidigm BioMarkTM HD System
- Juno System

Troubleshooting

Before start

- Wear protective gloves and lab coat while performing this procedure.
- Perform all open-vessel work within a laminar flow biological safety cabinet to avoid contamination of samples or operator exposure to potentially infectious materials.
- Dispose of all biological waste within the appropriate waste containers.

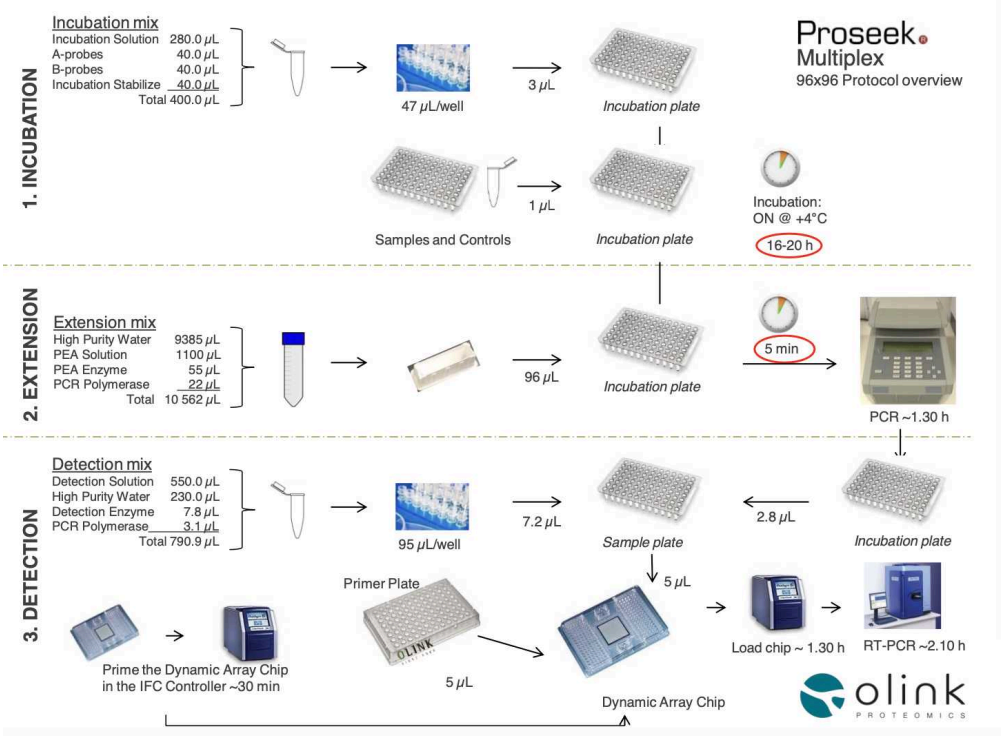
Sample preparation Notes

1 The serum and plasma samples for the Olink Inflammation and Immuno-Oncology panel are run neatly. However, every panel has different Sample Preparation requirements for different sample matrices. For example:

- Serum and Plasma samples for the Olink CVD III panel should be diluted 1:100 using the Olink sample diluent.
- Human Breast Milk samples for the Olink Inflammation panel should be diluted 1:2 with Olink sample diluent. The samples must be prepared following the SOP HIMC-4042: PREANALYTICAL PROCEDURE OF HUMAN BREAST MILK SAMPLES FOR OLINK ASSAY.

Overview of the assay

2



Day 1: Sample incubation with Probes

16h 5m 10s

3 Important Note: All the vortex steps require 00:00:30 vortex time, except for the Enzyme and PCR polymerase. 30s

4 Thaw samples, vortex and spin down at 400 x g, Room temperature, 00:01:00 1m

5 Thaw the Incubation Stabilizer from the Proseek Multiplex Controls box at room temperature, vortex for 00:00:30 and spin briefly, then place On ice . 30s

6 Thaw the interplate control (IPC) and negative control from the Proseek multiplex controls box in Room temperature , vortex 00:00:30 and spin briefly, place On ice . 30s

Prepare an 8-strip tube and mark the negative control on the top. Add 3×5 µL Negative Control and 3×5 µL IPC in an 8-strip tube (Figure 1).

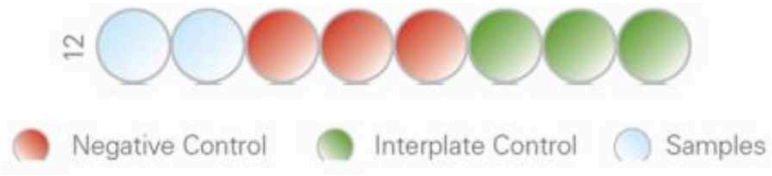


Fig 1 Add NC and IPC in 8-strip tube

7 Prepare incubation mix in 1.5mL microcentrifuge tube, vortex 00:00:30 and spin each reagent before transfer to the mix, pipette the incubation solution carefully to avoid foaming. 30s

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

8 Vortex the incubation mix for 00:00:30 and spin down the content. Transfer 47 µL per well of the Incubation mix to a new 8-well strip. Vortex the strip tube for 00:00:10 and spin down. 40s

9 Prepare a 96-well plate and label as Incubation plate. Mark the control position on the plate according to plate layout (Figure 2).Use a multi-channel pipette to transfer 3 µL of the Incubation mix from the 8-well strip to the bottom of each well of a 96-well plate by using reverse pipetting. Pipette from the uppermost part of the Incubation mix to prevent liquid from sticking to the outside of the pipette tip. Do not change pipette tips.

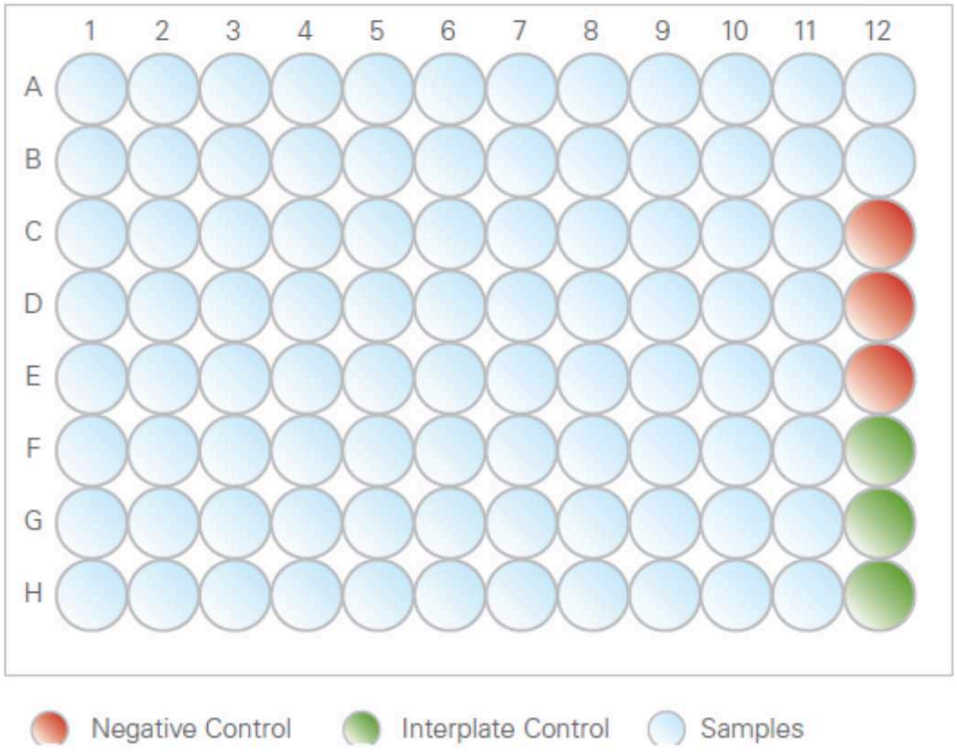


Figure 2. Incubation plate layout

10 Use amulti-channel pipette to add 1 µL of each sample to the bottom of thesample well of the incubation plate, according to the plate layout in Figure 2.

11 Use a multi-channel pipette to add 1 µL of negative control and IPC from 8-strip to the bottom of the well in position C12-H12, according to the plate layout in Figure2.

12 Seal the incubation plate with an adhesive plastic film. It is important that all wells are properly sealed, especially around the edges, to avoid evaporation of samples. Vortex the incubation plate for 00:00:30 and Spin down the content at 400 x g, Room temperature, 00:01:00 1m 30s

13 Incubate the Incubation Plate overnight at 2 °C to 8 °C. Be consistent for the incubation time at 16:00:00 16h

Day 2: Extension of the Conjugated Oligonucleotide 1h 48m 40s

14 Turn on your thermal cycler (HIMC Bio-Rad T100 Thermal Cycle) and create a PEA (Proximity Extension Assay technology) program on the thermal cycler with the following conditions:
Preheat the PCR machine by running this protocol and pause when the sample temperature reaches to 50 °C

Extension	50°C	20 min	
Hot start	95°C	5 min	
PCR Cycle	95°C	30 s	×17
	54°C	1 min	
	60°C	1 min	
Maintain the reaction at	10°C	∞, hold	

15 Thaw the PEA Solution, vortex, and spin briefly. Prepare the following extension mix in a 15ml Falcon tube. Use an ice box when removing the PEA Enzyme and the PCR Polymerase from -20 °C. Vortex 00:00:10 and spin down the content briefly before pipetting the enzymes into the mix. Keep the leftover of the PCR polymerase in the ice box, which will be used for the further step. 10s

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

16 Vortex the Extension mix for 00:00:30 30s

17 Bring the Incubation Plate to room temperature and spin down at 400 x g, Room temperature, 00:01:00 1m

18 Pour the Extension mix into a multi-channel pipette reservoir.

19 Carefully remove the plastic adhesive film from the Incubation Plate.

20 Steps 20 - 23 have to be performed within 00:05:00 . 5m

Start a timer before transferring 96 µL of Extension mix to each well of the Incubation Plate by using reverse pipetting. Do not change pipette tips, place the tips against the upper parts of the well wall.

21 Make sure the tips never come in contact with the contents of the wells.

22 Add a new aluminum seal film to the Incubation Plate; It is important that all wells are properly sealed, especially around the edges to avoid evaporation of samples

23 Vortex the plate thoroughly for at least 00:00:30 and ensure that all wells are mixed. Spin down the content at 400 x g, Room temperature, 00:01:00 1m 30s

24 Stop the PCR machine which was paused before, and wait the sample temperature to cool down to 30 °C . After the 00:05:00 timer done for previous step, place the Incubation Plate in the preheated thermal cycler and run the PEA program. This program will be approximately 01:40:00 1h 45m

25 Continue with the Detection step or store the Incubation Plate for up to one week at 4 °C

Detection of targeted oligos throughout real-time PCR 4h 25m 20s

26 Turn on the “Juno System” and verify that the HX interface plate has been correctly installed. Otherwise, change the Interface plate according to the following instruction: Tap “Tools” and “interface plate” bottom in the screen and select “Switch interface plate”. Place the HX interface plate and the loading fixture on the tray and tap “Install”. After the interface plate is installed, remove the loading fixture and tap “Close”

27 Take a new 96.96 Dynamic Array IFC. Inject control line fluid into each accumulator on the chip. Remove and discard the black protective film from the bottom to the chip. Place 50m

the chip into the IFC controller then run the “Primer” script. The Primer program will takes approximately 00:20:00 . Chip needs to be loaded within 00:30:00 after completed Primer, otherwise chip need to be primer again

28 Thaw the Primer Plate (offered in the kit) at 0 °C , Vortex the plate for Room temperature after thawing.

29 Thaw the Detection Solution, vortex 00:00:30 and spin briefly; prepare the following Detection mix in a 1.5ml microcentrifuge tube; vortex 00:00:10 and spin down Detection Enzyme and PCR Polymerase briefly before pipetting the enzymes into the mix. 40s

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

30 Vortex 00:00:30 of the Detection mix and spin briefly; transfer 95 µL of the Detection mix per well to an 8-well strip. Vortex the strip tube for 00:00:10 and spin briefly. 40s

31 Label a new 96-well plate as “Sample Plate”; use amulti-channel pipette to transfer 7.2 µL of Detection mix to each well of a new 96-well plate by reverse pipetting.

32 Remove the Incubation Plate from the thermal cycler; vortex the plate for 00:00:30 and spin down the contents. 30s

33 Carefully remove the plastic film and transfer 2.8 µL from each well of the Incubation Plate to the Sample Plate.

34 Seal the Sample Plate with a new plastic adhesive film, vortex the plate for 00:00:30 and spin with Primer Plate at 400 x g, Room temperature, 00:01:00 1m 30s

35 Gently remove the Primer Plate sealing to avoid contamination between wells; transfer 5 µL from each well of the Primer Plate to the primed 96.96 Dynamic Array IFC by using reverse pipetting; change pipette tips after each primer; Primers are loaded into their respective inlets on the left side of the chip according to Figure 3.

36 Transfer 5 µL from each well of the Sample Plate into the inlets on the right side of the chip according to Figure 3 by reverse pipetting; change pipette tips after each sample; See Figure 3 for a detailed instruction on sample loading.

37 Remove any visible bubbles, e.g., with a syringe needle.

38 After the samples and primers are loaded in all wells, place the chip with barcoding facing you in the IFC Controller for loading. Select “load” and “load mix 96.96 GE IFC” followed by “Run Script” to load the assay and sample mixes into the central portion of the Dynamic array. The Program will take approximately 01:32:00 1h 32m

39 Press the on/off button (on the right side of the instrument) to turn on the Biomarker and press the round button (on the left side) to connect the computer.

40 Open the “Biomarker Data Collection” and select the “start a new run” on the main menu, the lid will open and the tray come out on the Biomarker.

41 After the “Load mix 96.96 GE IFC” program is done, “Eject” the chip from the “Juno” system and remove the tiny dust from the Central Portion using the cello-tape, do this even though no visible dust. Take a piece of tape and let the sticky part touch (do not press down) the surface of the chip.

42 Load the IFC chip into the “Biomarker” tray with the barcode facing outwards and Select “Load”, the chip is now loaded into the instrument.

43 Select “Next” once the barcode and chip type is read and visible in next page. Select the “This is a new chip run” on this page and name the plate using HIMC format Then press “Next”

44 Set the following options for application reference and probe setting according to Figure 4.

Please select the following:

- Application: Gene Expression
- Passive Reference: ROX
- Assay: Single Probe
- Probes: FAM-MGB

Then select “Next”, and start run.

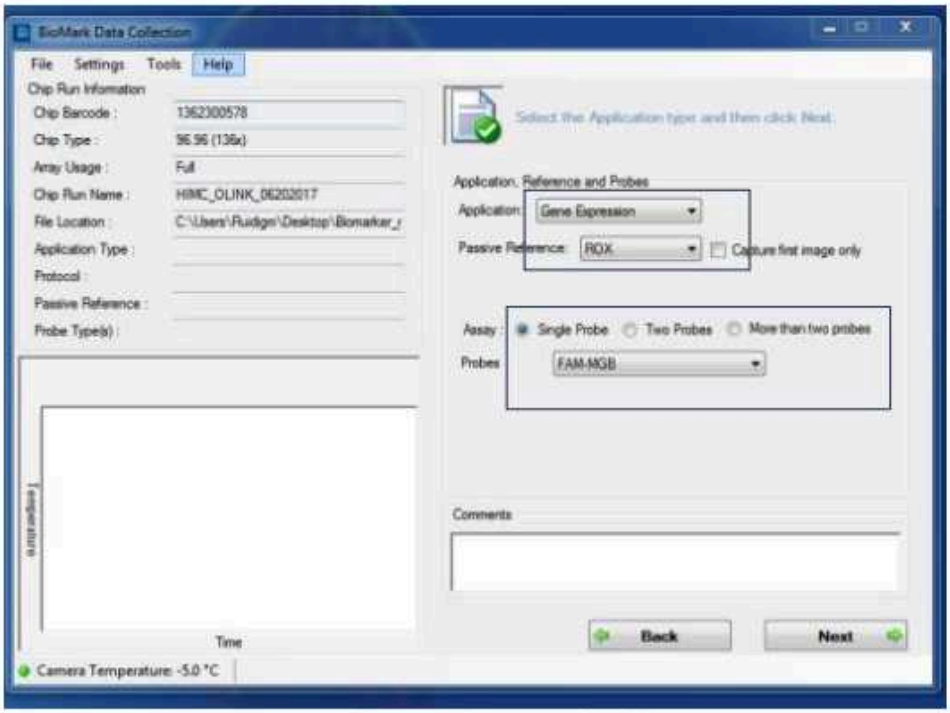


Figure 4 Application reference and probe setting

45 Select the “Olink_PE_96X96_New_35_03072019.pcl” protocol from the Olink folder on the desktop. Verify the protocol according to Figure 5. Then “Next”

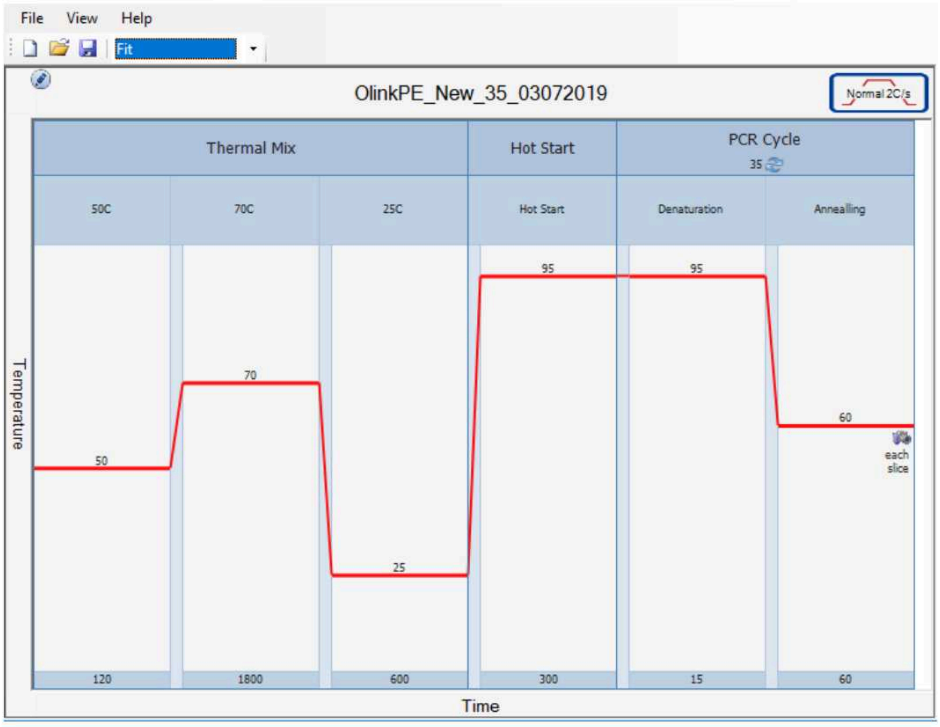


Figure 5 Olink Protein Expression 96x96 Program

46 Press “Start run” to start the program. The PE Program will take approximately 02:00:00 . There will be a message on screen “The Run has been successfully completed” after Run is finished. 2h

47 “Eject” the Chip from the Biomarker instrument.

48 Use an OLINK assay dedicated Flash Drive, and copy the study whole Folder with the Run name on the Flash Drive. Eject the flash drive.

49 If not running any more Chips on Bio-Mark on the same day, close the Bio-Mark software, shut down the Computer, and turn off the Bio-Mark machine power button.

50 Follow the SOP “HIMC-4031_R2_OlinkPEA_DataAnalysis”.

Protocol references

REFERENCE-Proseek® Multiplex 96×96 Kit, User Manual (http://www.olink.com/products/document-download-center/)