Jun 25, 2025 Version 2

CosMx for FFPE V.2

DOI
https://dx.doi.org/10.17504/protocols.io.3byl41mrolo5/v2

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Protocol Citation: Joanna Bi 2025. CosMx for FFPE. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl41mrolo5/v2Version created by **Joanna Bi**

Protocol status: Working
We use this protocol and it's working

Created: June 25, 2025

Last Modified: June 25, 2025

Protocol Integer ID: 220950

Keywords: cosmx for ffpe cosmx protocol, ffpe cosmx protocol, ffpe tissue, cosmx, ffpe, tissue

Abstract

CosMx protocol for FFPE tissue

1 FFPE blocks were sectioned into 5 µm sections using a microtome, placed onto BOND Plus Slides (Leica, SKU S21.2113.A), and dried as instructed prior to use (MAN-10159-02, NanoString). Sections were positioned within the imageable area as defined in the manual.

2 Dried sections were stored at 4°C until use.

3 Slides were baked at 60°C for 18 hours.

4 The FFPE sections were then deparaffinized and subjected to antigen retrieval at 100°C using Bond Epitope Retrieval Solution 2 (Leica, AR9640).

5 Incubation frames were assembled on the slides, and sections were permeabilized with Proteinase K at 40°C for 30 minutes, followed by a 5-minute fiducial incubation.

6 Post-fixation was carried out using 10% NFB, followed by Tris-Glycine treatment and blocking with NHS-acetate.

7 The sections were then incubated overnight with the CosMx Human Universal Cell Characterization Panel, which consists of a 950-target probe panel and 10 controls (NanoString, cat# 121500002).

8 After incubation, sections were washed to remove excess probes.

9 Cell membranes were then stained using morphology markers including CD298, PanCK, CD45, and CD68 from the CosMx Segmentation and Supplemental Markers Kit (NanoString, cat# 121500020, 121500021, 121500022).

10 Prepared slides were loaded onto the CosMx SMI instrument for imaging, following the NanoString manual (MAN-10161-02-1, Software Version 1.1). After pre-scanning each slide, regions of interest (ROIs) were selected as fields of view (FOVs) for further characterization.

11 The acquired scan data was then uploaded to NanoString's cloud-based analysis platform, AtoMx, for downstream processing and analysis.