

10x Visium Spatial Transcriptome

Q: How can we start the Spatial project?

A: We suggest that you schedule a consultation meeting with us before you start your project. This helps us better understand the goals of your experiment and determine which assay you will process.

Q: How to prepare the samples?

A: Please refer to 10X protocol, Visium HD FFPE Tissue Preparation Handbook, CG000684 | Rev D.

https://cdn.10xgenomics.com/image/upload/v1756507543/support-documents/CG000684_VisiumHDFPE_TissuePrepHandbook_RevD.pdf

Or refer to 10X protocol, Visium HD 3' Fresh Frozen Tissue Preparation: CG000804 | Rev B

https://cdn.10xgenomics.com/image/upload/v1756505803/support-documents/CG000804_Visium_HD_3_Fresh_Frozen_Tissue_Preparation_Handbook_Rev_B.pdf

Q: Do you have the RNA QC for FFPE samples and Fresh Frozen samples?

A: Yes, we provide RNA QC for FFPE samples and Fresh Frozen samples.

RNA QC assessment (DV 200): Please cut one or two FFPE tissue section with 10µm, put it into 1.5ml Eppendorf tube. We can process RNA extraction and TapeStation run to obtain the DV 200 value. If the DV 200 is higher than 30%, the same FFPE block can be used for Visium HD study.

For fresh frozen samples, RNA QC assessment (RIN): cryosection 20-30 mg of tissue sections from OCT-embedded tissue block (~4 sections at 25 µm thickness) put them into 1.5ml Eppendorf tube, freeze with dry ice. Transfer the tubes on dry ice to ICBR GE for RNA isolation and QC. The RIN should be higher than 7 to proceed.

Q: How thick of the tissue section?

A: For FFPE samples, 10x recommend 5 µm sections, but based on internal testing, 3-10 µm sections are compatible with the assay.

For fresh frozen samples, 10x recommend 10 µm sections.

Q: How to submit the tissue slides?

A: For FFPE samples, dry the Slide at 42°C for 3 hours and store the slide in a desiccator at room temperature for at least 12 hours.

- A. After drying, slides can be shipped in a slide mailer with desiccant pouches. Ensure that the slide mailer generates a tight seal. Transfer the tissue slides to ICBR GE Or store the tissue slide containing dry tissue sections at **room temperature** or **4°C** in a desiccator for up to **6 months**.
- B. Include ice packs if drastic temperature changes are anticipated during transportation.

For fresh frozen samples, make sure the tissue section is not thawing and section the 10µm tissue to place on the tissue slides. Freeze the section on cryobar and transfer it to a pre-cooled slide mailer or 50ml centrifuge tube. Prepare at least two replicates for each sample. Store the slides at **-80°C** for up to **4 weeks** or transfer the tubes on dry ice to ICBR GE.

Q: How will a high-quality image be obtained?

A: High-resolution H&E images and CytAssist post-transfer images are essential for accurate spatial mapping.

- Visium v1 for Fresh Frozen: 10x or 20x magnification
- Visium v2: 10x or 20x magnification
- Visium HD: At least 20x magnification (H&E); 10x magnification or higher for IF

An H&E resolution of 0.25–0.5 µm/pixel is recommended, which we achieve using the Olympus VS200 scanner from the Molecular Pathology Core (MPC)