

RNAscope and Basescope Sample Preparation Guideline for Fixed Frozen Tissue

1. Perfuse tissue with freshly prepared 4% paraformaldehyde (PFA) in 1X PBS. Alternatively, dissect tissue and place in freshly prepared 4% PFA for 24 HRS at 4°C.
2. Transfer and immerse the tissue to 10% sucrose in 1X PBS at 4°C until the tissue sinks to the bottom of the container (Note: the time needed for the tissue to sink varies with tissue type and size)
3. Transfer and immerse the tissue to 20% sucrose in 1X PBS at 4°C until the tissue sinks to the bottom of the container.
4. Transfer and immerse the tissue to 30% sucrose in 1X PBS at 4°C until the tissue sinks to the bottom of the container.
5. Freeze the tissue in OCT embedding media with crushed dry ice.
6. Store the tissue blocks at -80°C.

7. When ready to proceed, section the blocks on a cryostat by cutting sections to a thickness of 7-15 μm . Mount the sections on SuperFrost Plus Slides (Fisher Scientific #12-550-15). **Mount ONLY one section per slide at the center of the slide.**
8. Air dry the slides for 2 HRS at -20°C and overnight at -80°C. If not all the slides are used immediately, store them at -80°C for up to 3 months.