GeoMx DSP Protein and RNA Sample Prep Guidelines

When preparing, sectioning, and storing FFPE blocks for use in the GeoMx DSP instrument Protein and RNA assays, care should be taken to preserve sample integrity in all steps. The integrity of FFPE samples can be impacted by many factors, including time from excision to fixation, storage, tissue type, and sample age. It is important to take such factors into consideration when selecting samples for the GeoMx DSP assay. Samples with poor integrity are likely to give low signal, particularly in the RNA DSP assay.

Selecting FFPE blocks

FFPE blocks should meet the following criteria for the best performance with the GeoMx DSP assay.

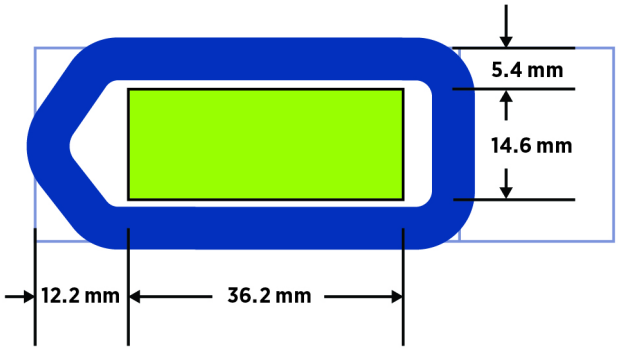
1. Blocks should be fixed in 10% neutral-buffered formalin for 18 to 24 hours at room temperature. This applies to tissues less than 0.5 cm in thickness. Larger tissues have not been tested by NanoString and may require longer fixation times.
2. Tissues should be fixed immediately after excision for best results. Up to one hour post-excision is acceptable.
3. Tissues should be thoroughly dehydrated in ethanol gradients prior to embedding in paraffin
4. FFPE blocks should be stored at room temperature, in a desiccator if possible.
5. For best results, do not use of FFPE blocks that are greater than 10 years old.

Sectioning FFPE blocks

For both the protein and RNA assays, it is important to avoid any scratches and folds in the section. These scratches and folds can be magnified by the subsequent slide washes on the GeoMx DSP instrument resulting in tissue loss. The following are general guidelines for sectioning FFPE blocks for optimal GeoMx RNA DSP assay performance. This is not meant to be an all-inclusive guide on sectioning. Please refer to your local pathologist or core for training on sectioning.

1. Sections should be cut at 5 µm thickness on a calibrated microtome.
2. Always discard the first few sections from the block face.
3. Sections should be mounted in the center of the slide to allow room for the gasket on the GeoMx slide holder.
    - NanoString recommends the use of SuperFrost™ Plus slides.
    - If mounting multiple sections per slide, ensure that all tissues are at least 2–3 mm apart.

For Slide Preparation, unstained tissue sections should be **5 µm** thick on **SuperFrost Plus slides**. Tissue sections **must be placed in the Scan Area** (the green area in the slide diagram) in the center of the slide and be **no larger than 36.2 mm long by 14.6 mm wide**. They should not overlap the slide gasket or the Tip Calibration area (this is the triangular region to the left of the green scan area in the slide diagram). If sections are larger than this size and/or placed off-center, it is likely that the tissue located outside the Scan Area will not be measured by the GeoMx DSP instrument.



- For DSP processing, ensure that **no hydrophobic pen or tissue** is in the **Scan** or **Tip Calibration areas** before placing your slides in the DSP slide holder.
- **Bake sections on slides** in a 60°C drying oven for a minimum of 30 minutes prior to deparaffinization. Longer baking times may be necessary for some tissues to adhere to the slide; this should be empirically tested. If tissue falls off, then baking longer *could* help.

> **NOTE:** In the event that sections are larger than the indicated size and/or placed off-center, continue with the slide preparation as usual. Just before loading the slide in the instrument slide tray, scrape parts of the tissue exceeding the scan area, making sure the slide gasket and tip calibration area are free of tissue. Scraping off tissue before the slide preparation could generate tissue folds that may result in staining/binding artifacts, while suboptimal scraping may result in leaks in the hydrophobic barrier.

4. Mounted slides should be allowed to air dry overnight. Store slides in a vertical position such that any remaining water can drain away from the tissue section
   - Any water trapped under the wax or tissue section should be removed by gently touching a folded kimwipe onto the corner of the wax section. The kimwipe should not contact the tissue.
   - It is recommended to use mounted sections within two weeks for best results. Older sections (1-2 months) may produce reasonable results, but this may be tissue or block dependent and should be tested empirically. Slides should be stored at room temperature in a desiccator or at 4°C prior to processing.

Selecting fresh frozen blocks

1. Tissues should be selected that are known to have been snap frozen in liquid nitrogen as quickly as possible.
   - Any buffers used to wash or temporarily store tissues before fixation should be free of nuclease contamination.

2. Frozen tissues should be embedded in Optimal Cutting Temperature media (OCT) before sectioning.
3. Blocks embedded in OCT should be stored at -80°C.

Sectioning fresh frozen blocks

1. Sections should be cut at 5 µm thickness on a calibrated cryostat and mounted immediately on a Superfrost™ Plus slide.
2. Sections should be centered and away from the gasket as shown above.
3. Always discard the first section from the block face.
4. After sectioning, the exposed block face should be covered with OCT to avoid desiccation of the sample.
5. Slides can be stored at -80°C for several weeks before use.