Application Note ♦ Working with RNA

Tips for working with RNA:

1. Freeze samples immediately after harvesting tissues using cryo embedding medium or freeze in liquid nitrogen.
2. Wear clean disposable gloves throughout the whole procedure.
3. Use RNAse-free instruments, and clean your work surface.
4. Samples should be savely stored in a freezer to prevent any thawing of the samples.

CHECK THE QUALITY OF YOUR SAMPLE STARTING MATERIAL BEFORE DOING ANY DOWNSTREAM APPLICATIONS; THIS SAVES BOTH TIME AND MONEY.

To check quality:
Cut a section from a block of tissue, extract the RNA and check the quantity on a spectrophotometer or bioanalyser.

Sectioning

1. Wipe cryostat (roll bar, knife holder) down with 100 % ethanol.
2. Use a new disposable blade, or clean the blade with 100 % ethanol.
3. Use clean brushes and forceps
4. Allow the sample block to sit in the cryostat for 10 minutes at - 18 to - 20 °C before sectioning.
5. Use room temperature slides that are RNAse-free and cut tissue 5 - 10 µm thick
6. Keep slides in the cryostat in a cooled slide box
7. Section the amount of slides you will use that day. Alternatively, the slides may be frozen in - 80 °C until ready to use.
8. Immediately proceed to staining and microdissection.

Staining

1. Staining should be done in a hood.
2. Wear clean disposable gloves, and make sure your pipettes are clean.
3. Keep slides frozen or in a desiccator until ready to stain.
4. Stain only the number of slides you will use that day. Alternatively, keep them in a desiccator.
5. Make up new staining solutions in nuclease-free water in autoclaved glassware.
6. Pipette stain onto slides, this will make the staining solution last longer.
7. After staining, dehydrate slides completely and store in a desiccator until ready for microdissection.

Microdissection

1. Wipe entire work surface down with RNAse inhibitor.
2. Wear clean disposable gloves.
3. Have all necessary equipment (such as tubes, buffer, and slides) available near the work station.
4. Work quickly.
5. After microdissection, place extraction buffer in tube and invert to stabilize sample. Keep all samples at room temperature in buffer until you finish your microdissections.
6. Spin tubes gently to collect extraction buffer in bottom of tube.
7. Samples may be stores at - 80 °C at this step for a few months time.
8. Do not isolate samples until just before downstream application.

Isolation

1. Wipe all surfaces down with RNAse inhibitor
2. Wear clean disposable gloves
3. Isolate RNA just prior to downstream application
4. Store unused sample eluate at - 80 °C