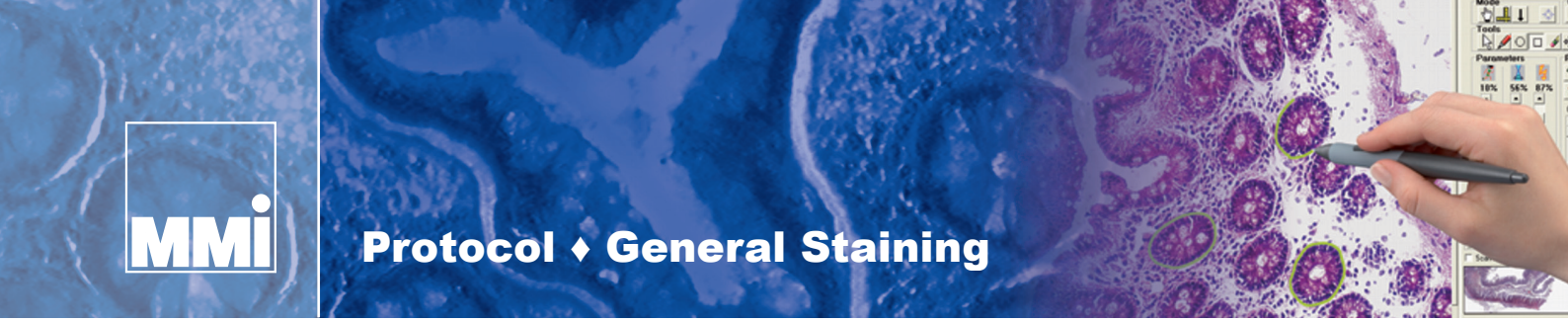

Protocol ♦ General Staining

When performing laser microdissection, it is necessary to be able to identify the cells of interest and differentiate them from the surrounding cells. In many instances, this will require using a histological stain. Many common stains are compatible with LMD and downstream analysis. However, before choosing a stain for a LMD experiment, the effects of the stain on the biomolecule of interest (DNA, RNA, Protein) should be evaluated. General staining protocols may need to be modified to preserve the biomolecules. The protocol below is a fast staining protocol and should work with most stains.

Materials:

- Xylene
- Graded ethanol (75 %, 95 %, 100 %)
- Pipette (~ 100 µl)
- Staining tray
- Copeland jars or similar
- RNAse-free water

Method:

For Frozen Tissue Sections:

1. Prepare seven copeland jars (or similar). Fill and label them:
   - 75 % EtOH
   - H_2O
   - H_2O
   - 75 % EtOH
   - 95 % EtOH
   - 100 % EtOH
   - Xylene
2. Remove sample (defined as a tissue section adhered to a membrane slide) from the - 80 °C freezer. Allow it to defrost at room temperature for 30 seconds.
3. Perform a quick fix of the sample by placing it in 75 % EtOH for 30 seconds.
4. Immediately transfer sample to H_2O for 30 seconds to hydrolyzes the sample and prepare it to accept the aqueous stain.

   Note: If using an ethanol-based stain, omit this step.

5. Place the sample on a staining tray or similar and pipette your stain (~ 50 - 100 µl) directly onto the tissue sample. Incubate for 10 - 30 seconds until desired staining quality is achieved.

   Note: The concentration of the stain may need to be modified to achieve desired staining quality.

6. Wash away excess stain by placing sample in H_2O for 30 seconds.
7. Begin gradual dehydration of sample by placing sample in 75 % EtOH for 30 seconds.
8. Place sample in 95 % EtOH for 30 seconds.
9. Finish dehydration by placing in 100 % EtOH for 30 seconds. At this point the sample is completely dehydrated and nuclease activity is minimal.
10. Remove ethanol by placing in xylene for 5 minutes.

    Note: If stain is negatively affected by xylene, omit this step. However, note that sample will absorb a small amount of water from the atmosphere; thus, RNase activity might slightly increase.

11. Air dry sample for 5 minutes to remove xylene.
12. Sample is ready for LMD.

    Note: Depending on inherent RNase concentration, RNase activity starts to compromise sample quality after 30 - 120 minutes.

For FFPE Sections:

1. FFPE samples can be stained in the same manner as frozen tissue sections, but must be deparaffinized and gradually rehydrated first.
2. Prepare seven copeland jars (or similar). Fill and label them:
   - H_2O, 2x
   - 75 % EtOH, 2x
   - 95 % EtOH
   - 100 % EtOH
   - Xylene
3. Stain sample according to the chart below:
   - Xylene - 3 minutes
   - Xylene - 3 minutes
   - 100 % EtOH - 30 seconds
   - 95 % EtOH - 30 seconds
   - 75 % EtOH - 30 seconds
   - H_2O - 30 seconds
   - Stain sample - 10-30 seconds
   - H_2O - 30 seconds
   - 75 % EtOH - 30 seconds
   - 95 % EtOH - 30 seconds
   - 100 % EtOH - 30 seconds
   - Xylene - 5 minutes
   - Air Dry - 5 minutes

   Note: If staining is too dark, reduce the staining time or dilute the concentration of the stain. If staining is insufficient, increase the staining time.