Preparation of frozen sections for the Leica LMD (laser microdissection)

General requirements:
Gloves must be worn at all times to avoid RNase contamination.

Materials and reagents required:
All reagents must be fresh to avoid RNase or proteinase contamination!!!

1. Ready-to-use slides with foil (Leica, cat. #11505128)
2. OCT embedding medium for frozen specimens
3. DEPC-treated distilled water
4. Ethanol solutions: 100%, 95% & 70% (70% diluted in DEPC-treated water)
5. Hematoxylin (Mayer’s or Harris)
6. Eosin
7. Aceton (optional)
8. 0.5 ml PCR tubes for microdissection (250 Eppendorf tubes, cat. # 0030 124.502)

Step I: Obtain membrane-coated glass slides (optional)

For destruction of RNases the slide is incubated for 30 min in a UV chamber (max. power). UV will improve the fixation of the foil.

Step II. Cutting of frozen sections for the Leica LMD

1. Embed tissue in OCT medium
2. Cut sections 4-20 µm thick on a clean cryostat with a clean blade
3. Eliminate folds and wrinkles
4. Mount on foil membrane as many sections as needed. If you have small piece, cut serial sections and mount the ribbon on the central part of the slide
5. Perform staining and LMD at the same day ASAP. Otherwise keep sections frozen at -20°C or for long term storage at -80°C.

Step III. H & E staining of frozen sections for the Leica LMD

1. Fixation: 70% Ethanol minimum 30 sec (RT) at -20°C (long term storage)
2. DEPC-treated distilled water wash 30 sec
3. Mayer’s hematoxylin 2 min
   or Harris hematoxylin 30 sec
   or Methylene blue 2 min
4. DEPC-treated distilled water wash 30 sec
5. Eosin 10 sec
6. 95% Ethanol 30 sec
7. 100% Ethanol 30 sec
8. Air dry (fume hood) appr. 5 min

NOTICE: Do all procedures gently; better to keep slides in horizontal position if possible.

*DEPC-treated water:
1) Add diethylpyrocarbonate (DEPC) at concentration 0.1% v/v to distilled water (1 ml to 1L),
2) Stir or shake,
3) Incubate for several hours,
4) Autoclave at least 45 min to get rid of DEPC.

Approved 06/2001, Dr. Maria Tretiakova, M.D., Ph.D.