Fixation, acetylation and dehydration

Materials:

1. PFA 4% solution[1]
2. 1x PBS solution[2]
3. 1x TEA buffer[3]
4. 0.9% NaCl
5. Acetic Anhydride (Sigma A-6404)
6. Cut tissue slides
7. Staining rack
8. Optional: Auto-Stainer
9. EtOH series, from 30% to absolute

Methods:

After you have sectioned the tissue you can proceed to post-sectioning procedures:

1. Fixation of the tissue. This step is done at room temperature.
    1. Put the slides into a slide holder
    2. Submerged the slides into the holder in 4% PFA 25 minutes
    3. Wash the slides with 0.9% NaCl two times for 2 minutes
2. Acetylation of the tissues
    1. Please the slide holder in a container and add 400 ml of 1x TEA buffer.
    2. Place the container on a magnetic stirrer. The solution should be stirred vigorously.
    3. Dispense with a pipettor 1ml acetic anhydride over the entire length of the container
    4. Move the slide rack in and out of the container vigorously 10 times
    5. Incubate for 5 minutes.
    6. After 5 min and repeat step 3,4 and 5
    7. Remove the tissue rack and proceed to Dehydration
3. Dehydration- drying slides in an ethanol series (to remove water)
    1. Dehydrate the tissues slides as follow:
       1x PBS 2 min
       0.9% NaCl 2min
       30% EtOH 2min
       50% EtOH 2min
       70% EtOH 2min
       80% EtOH 2min
       95% EtOH 2 min
       100% EtOH 2 min
       100% EtOH 2 min
       oven 30°C for 3 min
4. Slide storage

5. Place the slides in a small box in the presence of desiccant bags. Seal the boxes with electrical tape, label them and store at– 80°C.

Acetylation is an extremely critical step and determines background.

Notes:

1. 4% PFA solution preparation protocol- Paraformaldehyde in PBS (cshlp.org)
2. TEA buffer preparation protocol -TAE Buffer (50×) (cshlp.org)
3. PBS buffer preparation protocol- Phosphate-buffered saline (PBS) (cshlp.org)