



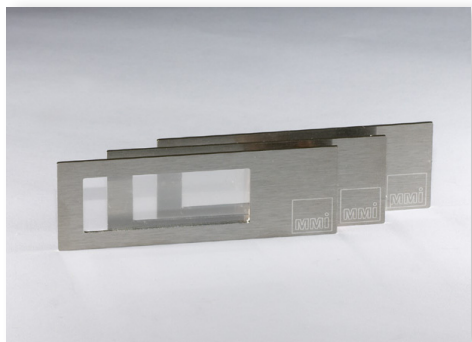
Protocol ♦ FFPE Embedding

This tutorial provides an overview of how to follow the Formalin Fixed Paraffin Embedded (FFPE) tissue process.

The goal for the sample preparation is to achieve precisely laser microdissected sample as well as high quality data for downstream molecular analysis of DNA, RNA, and proteins.

Materials:

- 10 % Neutral buffered formalin
- Paraffin wax
- Tissue processor
- Tissue embedding cassettes
- Microtome and related accessories
- Solvents and reagents necessary for paraffinization
- Dry incubation oven
- MMI Membrane Slides (PN: 50102, 50103)
- Water bath
- Nuclease-free water
- Necessary reagents to decontaminate work area



Method:

Tissue Acquisition and Fixation:

To avoid degradation of genomic material due to inherent nucleases, it is crucial to transfer the collected tissue to the fixative immediately after excision. The tissue sample size should be 0.5 x 0.5 x 1.0 cm at the maximum. Fixate for 8 - 16 hours in 10 % neutral buffered formalin.

Paraffin Process:

Once the tissue is fixed, complete the following steps of dehydration, clearing, and infiltrating with paraffin.

- Wet fixed tissues (in aqueous solutions) must be dehydrated with a series of ethanol (e.g. 70 %, 95 %, 100 %), steps 1 - 6
- Remove the dehydrator (Ethanol) with Xylene (or HistoClear) using the clearing steps 7 + 8
- Infiltrate the tissue with the embedding agent, paraffin (to aid penetration, apply vacuum inside the tissue processor), applying steps 10 - 12
- Applying the above steps, the moisture inside the tissue is replaced by hard paraffin and can be placed in a tissue embedding cassette containing more liquid paraffin - allow to harden

Sectioning:

- Use a microtome to cut tissue into thin sections between 5 - 10 μ m
- Float cut sections in warm, nuclease-free water bath to help remove wrinkles
- Use the MMI Membrane Slide to pick up the cut sections
- Place slide at an angle to allow water to drain and incubate sample at room temperature for 60 - 90 minutes (do not bake sample)
- Slides can now be stored at room temperature for future use

Note: Before staining and microdissection, the slides must first be de-paraffinized