Tissue Preparation : General information

Paraffin embedded tissues

- Wax tissue solidification technique enabling thin sections (4–5µm).
- Works on fixed tissues (10% Neutral buffered formalin)
- Not suitable (less) for labile antigens detection or DNA/RNA isolation (option: tissue freezing)

Formalin Fixed Paraffin Embedded (FFPE) Tissues preparation steps:

1. Sample should be dissected as soon as possible after euthanasia. You may choose a representative area of the whole tissue and avoid any compression to prevent tissue damage. Cut into thin slices using a clean scalpel and pay attention to organ orientation. Slices should be 3 to 5 mm thick.

2. Samples are placed inside histology cassettes before processing step. Special care should be taken to cassettes identification using a pencil. Please, do not use ink pens: ink identification on your cassettes will be cleared during xylene /alcohol processing steps.

3. Cassettes with corresponding samples will be stored inside sealed containers full of fixative (10% Neutral buffered formalin is recommended). Fixation will preserve tissues and keep them close from their native structure. Fixative volume should be 15 to 20 times higher then specimen volume. Fixation time will vary for small and big tissues. Fixation speed is about 1mm/H at room temperature.

4. Send your specimens stored in well sealed containers to our facility using a shipping service.
If you have any question or if you are unsure about your specimen integrity, do not hesitate to contact us. We will be pleased to help you depending your needs.

Good sampling and fixation make quite a difference in obtaining reliable results.

**Frozen Tissues**

Freezing (Temperature decrease up to −20°C or−80°C) will prevent from autolysis. Frozen sample can be cut into thin sections using a cryostat.

- Tissue solidification technique enabling same cutting procedures then FFPE samples, excepting embedding medium!
- Non fixed frozen tissues will demonstrate high antigenicity but poor morphology.
- Suitable for labile antigens detection and DNA/RNA preservation.
  
  ➔ Tissues are frozen and embedded in a gel (O.C.T.) which solidifies at −20°C. OCT will enable cellular ultrastructure and nucleic acids preservation.
- Not suitable for studies requiring optimal morphology (option: paraffin embedding)
  
  ➔ Freezing can be performed on fixed tissues or fresh tissues (no chemical fixation)

- Fresh tissues are easy to cut. Nevertheless adipose tissue will be easier to cut after fixation.
- Fixed tissues:
  - 10% Neutral buffered formalin or paraformaldehyde
  - Mercury based Fixatives (not recommended)
  - Alcohol based Fixatives (will inhibit freezing)
Steps for frozen tissues preparation

**Fresh tissues**

1. Tissue dissection thickness shouldn't exceed 3 mm while its length should fit with disposable plastic Cryomolds. (Different sizes are available in our facility if needed. Do not forget to identify properly each sample with a permanent marker or with an additional filled paper which will be folded inside aluminium foil wrap)

2. **Isopentane** and **dry ice** should be placed inside a large container (glass dish) to enable temperature drop. Recipient cryomold should be put in the middle of container. Dry ice may not touch cryomold to avoid any blasting. Place sample inside cryomold and apply OCT immediately. OCT should cover all the sample. OCT will freeze in a few seconds thanks to temperature drop. Avoid air bubbles when applying OCT.

3. Store your samples at -80 °C or -20°C depending your project needs (routine processing or IHC/IF/ISH)

4. Send your specimen in an icebox or in a styrofoam box filled with dry ice to prevent from temperature deviations.

5. **Mouse/Rat Brain special care**
   Cool isopenthane up to -30° / - 35°C on dry ice and dip brain inside. Wait for 30 sec to 1mn, and wrap brain inside an aluminium foil above the dry ice. Store at -80°C for at least 48H before cutting specimen.
Fixed tissues

1. Sample dissection
2. Fixation in 10% neutral buffered formalin (check volume)
3. Specimen shipping inside a fixative sealed container to our facility.
4. Once delivered, specimen will be incubated in 25% sucrose for 2 hours until the tissue sinks (dehydration step enabling good cryopreservation = better morphology avoiding crystal formation during freezing), then tissue will be dried on a paper, and OCT embedded.

ATTENTION

Before sending all you samples to our facility, please take 5mn to fill the PDF service request form. You can contact Mrs Julie Hinsinger to get a copy.

Your request will be processed more easily by including any specific detail, special needs and updated contact information.
FFPE tissues processing steps

1- Preparation and cassetting
   a. Fixed tissue is sent to the Histology facility
   b. If not done yet, grossing, slicing and cassetting are achieved by our technical staff.

2- Processing : wax infiltration step inside tissue sample
   a. Dehydration : 3 alcohol baths with growing concentrations: 70%-85%-90%, preventing tissue damage (distortion, hardening). Water is finally removed by 3 final absolute alcohol baths.
   b. Clearing : 3 toluene baths will enable to replace alcohol trapped inside tissues and to be a miscible solvent with wax.
   c. Wax infiltration : Hot wax baths (44˚Cà 60˚C) will solidify the tissue.

3- Tissue embedding
   a. Tissue is orientated inside a mold filled with hot paraffin.

4- Tissue sectionning using a microtome
   a. Most of the time, histology sections are 4 µm thick. Wax ribbons are transferred onto a warm water bath (43-45˚C) and spread on a microscope glass slide before drying at least for one hour at 45˚C.
FFPE tissues processing steps

5- Routine Stain
   a. HE (Hematoxylin-Eosin) or HPS (Hematoxylin-Phloxin-Safran)
      Deparaffinize
      2 baths of toluene (95%-100%). 3 minutes each.
      3 baths of alcohol (80%-95%-100%). 3 minutes each.
      Rince in tap water
      Gill Hematoxylin for 3 to 10 minutes
      Sodium Bicarbonate for 5 to 10 seconds
      Rince in tap water
      Eosin/Phloxin 1 minute.
      Rince in tap water
      3 baths of absolute alcohol. 40 seconds each.
      (Safran. for 3 to 10 minutes)
      3 baths of absolute alcohol. 40 seconds each.
      3 baths of toluene

   b. Special stains :
      We do offer a wide range of special stain techniques. You can find more details on our price service list.

6- Pathologist analysis
Frozen tissues processing steps

1- Preparation and OCT embedding
   Tissue is sent fresh frozen or formalin fixed following our recommendations
   (details in tissue preparation: general information)

2- Cryostat sections
   a. Most of the time sections are 7 µm thick.
   b. Frozen sections are spread onto a microscope glass slide stored at room
      temperature with a brush which is kept at -20°C so that sample can easily
      stick to the slide.
   c. Cutting temperature won’t be the same for every tissues.

3- Routine stain
   a. HE (Hematoxylin-Eosin) or HPS (Hematoxylin-Phloxin-Safran)
      Same steps than paraffin routin stain with a shorter incubation time.

   b. Special stains
      We do offer a wide range of special stain techniques. You can find
      more details on our price service list.

4- Pathologist analysis