

# TCP Pathology Sample Prep Guide

1. Collect tissue immediately after euthanasia.  
*It's prudent to collect more organs and tissues than you anticipate needing and submit only those initially required. During histopathology analysis, other organs may be needed as a reference to confirm interpretations.*
2. Don't freeze animal carcasses. If tissue collection cannot be done immediately, immerse in 20x volume of 10% formalin. Refrigerate carcass as a last resort.
3. Avoid mechanical trauma.  
*Specimen should not be allowed to dry out prior to fixation. If immediate fixation is not practical, keep specimen wrapped in a piece of gauze moistened with saline.*
4. Avoid heat (e.g. cautery) and chemical (e.g. disinfectants) damage.
5. Fixation
  - a. Ensure prompt fixation  
*If immediate fixation is not possible, specimen should be refrigerated at 4 °C.*
  - b. Use sufficient fixative and a suitable container  
*An adequate volume of fixative (ratio of at least 20:1) is used in a container of an appropriate size. This avoids distortion of the fresh specimen and ensures good quality fixation.*
  - c. Check fixative is of high quality and at the optimal pH.
  - d. Bring samples in labelled (label the container not the lid), leak proof containers.
  - e. Fix tissues in flat bottomed jars, not conical tubes.
  - f. Avoid containers with narrow necks.  
*Tissues may expand in formalin making it difficult to remove through a narrow neck after fixation.*
  - g. Gentle agitation during the first 24 hours at room temperature in fixative will improve the quality of fixation.
  - h. Types of fixatives
    - i. Most tissues can be fixed in 10% neutral buffered formalin (NBF) (recommended).

- ii. 4% paraformaldehyde can be used, but rate of fixation is slower and it needs to be made up just before use as it is unstable.
- iii. For eyes, Davidson's fixative is recommended
- iv. For embryos, endocrine tissue, reproductive tissues, and brain, Bouin's fixative is recommended
- v. Avoid fixing mouse brain in ethanol or transferring mouse brain to alcohols after formalin fixation, to avoid a vacuolar artifact in the white matter.

i. Time.

*24 hours at room temperature on a shaker should be sufficient for most mouse organs (2-5mm thick, 2 cm in diameter). Tissues with larger surface areas (i.e. diameter of a quarter) should be fixed from 24-48 hours. Avoid extended periods of time (one week or longer).*

j. Size.

*Expose as much of the tissue to fixative as possible (cut sample in half or sections). Fixation proceeds from outside to inside, so cells near the surface will fix sooner than cells on the interior of the specimen. Cut in half organs that are encapsulated to allow the fixative to penetrate the centre of the tissue. In animals larger than a mouse (rats, rabbits, swine, etc.) many or all organs will need to be cut to no thicker than 5 mm. In a rat, kidney, liver, and testes need to be cut into smaller pieces before fixation.*

k. Temperature.

*Fix at room temperature. Fixing at 4°C slows fixation rate which can result in suboptimal staining.*

l. Post fixation solutions.

*After fixation, transfer to PBS or 70% ethanol for up to one week in refrigerator. For longer periods of time, add a small amount of sodium azide to prevent bacterial growth.*

6. Sample prep for tissue sectioning

- a. Tissues can be formalin fixed paraffin embedded (FFPE) or frozen.

**FFPE vs Frozen**

<i>Formalin Fixed Paraffin Embedded (FFPE)</i>	<i>Frozen</i>
<ul style="list-style-type: none"> <li>● stored at room temperature, which is convenient and cost-effective</li> <li>● works well for immunohistochemical staining and morphology analyses</li> <li>● formalin is toxic</li> <li>● fixation and paraffin-embedding</li> </ul>	<ul style="list-style-type: none"> <li>● fast</li> <li>● suited for molecular analysis (DNA, RNA and post-translational protein modifications (PTMs) using RNA free techniques)</li> <li>● required for oil red O staining for lipids</li> </ul>

procedures are time-consuming <ul style="list-style-type: none"> <li>generally unsuitable for molecular analysis</li> </ul>	<ul style="list-style-type: none"> <li>better if native morphology of protein, antigen or epitope is required, since conformation is not altered by fixation</li> <li>must be frozen as soon after collection as possible</li> <li>requires freezer space</li> </ul>
---	--

b. For frozen sections- tissues can be fixed or fresh before freezing. *See Protocols below.*

### Fixed vs Fresh

<i>Fixed</i>	<i>Fresh</i>
<ul style="list-style-type: none"> <li>better morphology</li> <li>better preservation for fast deteriorating epitopes</li> </ul>	<ul style="list-style-type: none"> <li>good for IHC, IF, ISH</li> <li>no antigen retrieval required</li> <li>often easy to section</li> <li>poorer morphology</li> <li>prone to freezing artifacts</li> </ul>

### Protocols:

#### A. Freezing Fresh Tissues for Frozen Tissue Sectioning

Solutions/materials:

- OCT or any freezing embedding media
- Cryomolds
- plastic wrap
- liquid nitrogen

Method:

1. All tissue must be fresh and with as little water as possible (any water will cause freezing artifacts).
2. Place tissue in a cryomold, with cut surface face down, and fill with OCT ensuring no bubbles are present.
3. Cover cryomold with one layer of plastic wrap and let it float on liquid nitrogen until frozen.
4. Store at -80°C until ready to cut frozen section.

Note: Sections must be placed on commercial coated slides. Air dry after thawing may also help sections to adhere to the slides better.

## **B. Fixing and Freezing Tissues for Frozen Tissue Sectioning**

### Solutions:

- Any aldehyde fixative
- 15% sucrose
- 30% sucrose

### Method:

1. Fix tissue in an aldehyde fixative for the appropriate amount of time.
2. Place tissue (<3mm) in 20ml solution of 15% sucrose overnight.
3. Then transfer the tissue in 20ml solution of 30% sucrose overnight (if tissue is still floating, incubate for 2 or more nights).
4. Blot tissue very well with Whatman filters until all moisture is removed.
5. Place in a cryomold, fill with OCT ensuring no bubbles are present.
6. Cover cryomold with one layer of plastic wrap and let it float on liquid nitrogen until frozen.
7. Store at -80°C until ready to cut frozen section.

Note: Sections must be placed on commercial coated slides. Air drying after thawing may also help sections to adhere to the slides better.