

**Tissue fixation for H&E.v1**

These are different procedures for tissue fixation for various morphological analyses. This protocol may not be compatible with immunohistochemistry for some antibodies.

**Tissue fixation of H&E and immunohistochemistry**

1. Perfuse the mouse with saline followed by the fixative if possible. This will give the best morphological preservation
2. Cut the tissues into a small piece and place it in an Eppendorf tube containing 10% neutral balanced formalin (actually concentration is 3.7%) or 4% paraformaldehyde on ice and then bring the tubes to cold room to rock overnight.
3. Wash the samples with PBS for 20 min at room temperature for 3 times.
4. Wash the samples in 50% ethanol for 2 hrs at room temperature.
5. Wash the samples in 70% ethanol for 2 hrs at room temperature.
6. Change fresh 70% ethanol and store the samples at 4°C.

**Preparation of 10% neutral buffered Formalin**

100 ml        Formalin (37-40% stock solution)

900 ml        ddH<sub>2</sub>O

4 g            NaH<sub>2</sub>PO<sub>4</sub>

6.5 g          Na<sub>2</sub>HPO<sub>4</sub>

Check pH around 7