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Fixation & Tissue Processing Protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

Fixation & Tissue Processing Protocol

Fixative

1

Note

* Freshly made RNase-free 4% paraformaldehyde (4% PF) used specifically for *In Situ* Hybridization

* 4% paraformaldehyde or 10% buffered formalin used for routine histology (i.e., H&E staining)

* 2% paraformaldehyde (bgal, GFP, RFP), 4% paraformaldehyde or 10% buffered formalin used for Immunocytochemistry

Fixation procedure

2

Note

The following protocols are for tissue sample processing for subsequent light microscopy with H&E staining, immunocytochemistry or in situ hybridization. RNase-free PBS and fixative should be used for in situ hybridization with RNA probes.

* Use the 20 ml scintillation vials with 15-20 ml solution volumes or
60 ml yellow-capped containers with 30-40 ml solution volumes to fix, wash and dehydrate the samples.

Well-fixed samples can be stored in the same fixative at 4˚C for several days or even longer for H&E staining. In situ hybridization samples must be dehydrated and stored at -20 oC with 100% ethanol after 24-48 hours fixation. Immunohistochemistry samples must be dehydrated and stored at -20 oC with 100% ethanol after overnight fixation.

Tissue fixation

3 Isolate the tissue into **cold PBS** as soon as possible (can be omitted).

4 Wash tissue with **PBS** to remove all blood (can be omitted).

5 Place tissue in **fixative** for 10-15 minutes to one hour (can be omitted)

6 Cut tissue to proper size. The size can be 2X2 mm to 1X2 cm but thickness should be 3 mm for better fixation. The cutting surface of the tissue should be flat and smooth.

7 Transfer tissue to **fixative** and swirl the container to ensure all tissues are completely immersed in fixative. The volume of fixative must be 20-30 times the tissue volume.

8 Fix tissue at 4˚C for overnight.

9 Check sample the next day to ensure proper fixation.

For best morphology and staining results take care to:

10

Note

1. Use high-quality fixative and sufficient amounts of fixative.
2. Use sharp razor blade and fine instruments such as scissors and forceps to handle all specimens. A piece of dental wax can be used as a cutting board. Treat tissues as gently as possible to minimize morphology artifacts.
3. Don’t let samples dry during preparation. Samples should always be covered by solutions.