Overview of solutions and procedures we routinely use for fixing and decalcifying teeth for irnmunohistochemistry

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Fixative: 4% paraformaldehyde + 0.1% glutaraldehyde in 0.08 M sodium cacodylate buffer + 0.05% calcium chloride. Adjust pH to 7.2-7.4 before using

40 g	paraformaldehyde
4 ml	25% EM grade glutaraldehyde (purified; obtain from EMS) sodium cacodylate
17.12 g	calcium chloride
0.5 g	
Make to 1 liter	

Fix by vascular perfusion at room temperature, remove jaws and immerse in fresh fixative for a few hours or overnight at 4°C. Rinse tissues after fixation in whatever washing buffer you prefer.

-You have to heat the paraformaldehyde to almost the boiling point in dH20 and add NaOH to get it to dissolve (let me know if you do not know how to do this step)

-This fixative only works well by vascular perfusion for rodents that are 10 days after birth or older (do not use this solution for immersion fixation, it will not be very good).

-This fixative, however, can be used for immersion fixation of rodent embryos and newborn (up to 10 days old). Best results are obtained by enhancing the immersion fixation through microwaving (e.g., see Laboux et al. (2004) J Histochem Cytochem 52:1267-1275).

-Some antigens will still work at glutaraldehyde concentrations up to 0.5% (the quality of tissue preservation improves dramatically).

-There are some cases where 1% glutaraldehyde alone (no paraformaldehyde) in the above solution will work. You have to increase the molarity of the cacodylate to 0.1 M.

-If you just want good morphology , we get excellent results perfusing with 2.5% glutaraldehyde in the calcium/cacodylate buffer or better yet with 5% glutaraldehyde in the calcium/cacodylate buffer (see any of our old morphology papers).

Decalcification (routine):

4.13%	disodiurn EDTA
41.3 g 4 .5 g	disodium EDTA (we use Sigma Aldrich brand) sodium hydroxide pellets

Adjust pH to 7.2 as required and make final volume to l liter distilled water
Decalcify at 4°C in a large container with agitation (e.g., 1 L flask with stirring bar)

Decalcifications take roughly 1 week per 30 gram rodent body weight (e.g., the incisors and molars of a 100gm rat take about 3 weeks to decalcify in this solution).

-The fluid volume to tissue ratio is very critical for the decalcification process. It should be as large as practical (e.g., no more than 10 or 15 hemi-jaws per liter of solution.

-You should change the solution every other day (pre chill the new batch to keep temperature at 4°C at all times).

-It is important to clean away as much muscle and soft tissues from the hemi-jaw bones; excess tissues act as a barrier to the decalcification process.

-We generally wrap each hemi-jaw in a separate gauze bag with an identifying label, tie off the top of the bag with some string from which the bag is suspended in the decalcifying solution.

-You have to wash the tissues very thoroughly after decalcification for at least 2 days at 4°C, changing the wash buffer frequent (e.g., at least 4 times a day). Use any buffer you like for washing as long as it is reasonably isotonic and has a pH around 7.2-7.4.

-Reference: Warshawsky and Moore (1967) J Histochem Cytochem 15: 542-549.

3. Decalcification (hurry up): Acidic aluminum chloride (be very careful with this solution)

12.61 g Aluminum chloride, hydrous (·6H20)
WARNING: never, ever use the anhydrous form of AlC13 which is explosive in this formula
8.5 ml 10 N hydrochloric acid 88% Formic acid
5.4 ml

Make final volume to 100 ml. Dilute 1 part of this stock solution with 4 parts of dH20 for LM histology (or immunohistochemistry) and 1:8 for tissues being processed for EM

-This is a "black magic" decalcification technique I stumbled on many years ago when I was a graduate student that very few people know about other than Antonio . The aluminum ions in this solution break up mineral very rapidly by nucleophilic attack. Although highly acidic, the tissues hold up pretty good but only in diluted solution. Never use the concentrated stock as it will destroy the nuclei and chew up the tissue.

-The technique is based on a paper published in 1952 in German by J. Plank and A. Rychlo in a journal called Zentrablatt fur Allgemeine Pathologie und Pathologische Anatomie 89: 252-254. I have a Xerox copy and translation if you are ever interested.

-Decalcification of an entire head of a 100 g rat takes only about 5 days in this solution at 1:4 dilution. It is truly amazing how quickly it works and how reasonable the tissues look afterwards.

-We usually do the decalcification at 4°C as with EDTA (with stirring, large fluid to tissue ratio). You should change this solution daily. Wash the tissues liberally after decalciftcation as with EDTA