Recipes: buffers, fixatives, etc

0.1M Phosphate buffer solution

- Prepare 4 volumes of 0.1 M sodium phosphate dibasic (Na2HPO4), i.e. 800ml for a 1l solution.
- Prepare 1 volume of 0.1 M sodium phosphate monobasic (NaH2PO4), i.e. 200 ml for a 1l solution.
- Mix 4 parts of the dibasic solution with 1 part of monobasic to make a final 0.1M phosphate solution.
- Adjust the pH to 7.3-7.4

0.1M Phosphate buffer solution + 0.3% Triton-X

- Use the phosphate buffer made as indicated above and add Triton-X to a final concentration of 0.3% (i.e. 0.3 ml in 100 ml solution).

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Bouins fix

Preparation (for 1 liter):

- Measure 750ml filtered saturated picric acid
- Add the picric acid to 250 ml of 40% formalin
- Add to the mixture above 50ml glacial acetic acid
- Stir until mixed

Store at room temp; stays good forever.

Notes:

- ALWAYS put the date on a freshly made solution. Also add your initials, pH and concentration.
- Label anything that touches fixative solution with the words FIX (or something like that). Dishes or stirrers once used for fix should not be used to make non-fix solutions such as salines!!!

Recipes: buffers, fixatives, etc

4% Paraformaldehyde solution

Preparation (for 1 liter):

- Prepare 4 volumes (800 ml for 1l) of 0.1 M sodium phosphate dibasic (Na2HPO4).
- Prepare 1 volume (200 ml for 1l) of 0.1 M sodium phosphate monobasic (NaH2PO4).
- Warm the dibasic phosphate solution to 60°C.
- Weigh the paraformaldehyde (i.e. 40g for a 1l solution, 4g for a 100ml solution).
- Add to the warm dibasic phosphate stirring constantly (do not exceed 70°C; if you do, then start again).
- When paraformaldehyde has disolved (should take ~ 5min) add the monobasic phosphate solution.
- The pH should be about right, but check it. If not correct, then add NaOH or HCl to bring it to 7.4.
- If the solution has been heated for too long, white flakes may appear. If there are a lot, start again. If there are only a few, filter the solution.
- Add 400mM sucrose (this is to adjust the osmolarity of the solution to something close to the saline's).

Troubleshooting:

- Paraformaldehyde (the powder) reacts readily with oxygen and oxidizes to a less active form. Thus, in order to preserve it in the best possible condition it should be weighed in aliquots (2 or 4 grams, for example), packed tightly to eliminate as much air as possible (ideally in a N2 atmosphere), sealed in small container and stored frozen.
- Paraformaldehyde solutions go bad rapidly. Thus, ALWAYS put the date on a freshly made solution, and throw the solution away after 1-2 days.
- Label anything that touches fixative solution with the words FIX (or something like that). Dishes or stirrers once used for fix should not be used to make non-fix solutions!!!

Alternatively, use premade Paraformaldehyde from Electron Microscopy Science and dilute it with phosphate buffer.

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15% Picric acid + 2% Paraformaldehyde fix

Preparation:

- Carefully heat up 15ml double filtered saturated picric acid to 60°C.
- Add 2g paraformaldehyde (it will get all thick and cloudy).
- Slowly add strong NaOH (5N) until everything is in solution. "Clear" is when you can't see any more particles in the picric acid. The original recipe calls for 2.52% NaOH but it does not have to be exacly 2.52%.
- Filter again and fill to 100ml with phosphate buffer and keep at 4°C in a dark bottle.
- Make sure pH is ~7.3.

Note:

- ALWAYS put the date on a freshly made solution. Also add your initials, pH and concentration.
- Label anything that touches fixative solution with the words FIX (or something like that). Dishes or stirrers once used for fix should not be used to make non-fix solutions!!!

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Ice-cold ethanol fix

Preparation (for 1l):

- Prepare 95% Ethanol solution. Ethanol can be bought at a concentration of 100% percent, which is called anhydrous or denatured. In this case it has to be diluted to 95%. It can also be obtained at 95%. 95% is the highest concentration that ethanol can be destilled to. To make it completely free of water a chemical process is used that may add traces of chemicals to it. So the best is to simply get destilled ethanol. It is also much cheaper.
- Cool it to about 0°C (placing it in ice water is the most effective way).
- Rinse your preparation a few times with this ethanol solution and then leave it on ice for 1-2 hours. Depending on the thickness of the tissue. For STGs it seems to work OK if fixed for 1 hour. If you leave it on ice, make sure that the ice is wet so that the temperature will indeed be kept at ~0°C, but make sure that your dish does not sink into the ice (which may happen if you are using the dissection Petri dish for this).
- Rinse 2-3 times every 60 minutes in phosphate buffer + Triton X (if you are proceeding with immunostaining) or plain phosphate buffer if you are doing something else.

Keep your Ethanol solution sealed to prevent Ethanol evaporation. Since Ethanol and water evaporate at different rates, the EtOH concentration can change quite a bit. The tissue becomes white and hard in EtOH but becomes translucent and soft again when washed in phosphate buffer. It tends to stick to the walls of the plastic containers, etc. much more than when using aldehyde fixes.

ALWAYS put the date on a freshly made solution. Also add your initials and concentration.

With this particular fix, you can use your standard physiology dishes and tools. EtOH evaporates and and very low concentrations is completely harmless.

Recipes: buffers, fixatives, etc

Stainless Steel solder

Solution good for soldering stainless steel, tungsten, nichrome, possibly even niobium. It really cuts through oxides. CAUSTIC, CORROSIVE. Wear safety glasses, etc.!!!

Used to make non-corroding electrodes (stainless steel) corrosibles.

Important:Use ORGANIC CORE SOLDER. Do NOT use the standard rosin core solder (the one that has a resin core and used for tin soldering). This one contains an organic (water soluble) core.

Jesse's Super Flux (Don't ask who Jesse is!):

- 96g $ZnCl_2$
- 9.6g NH_4Cl
- 25ml H_2O
- 10ml HCl (conc.)
