# PBS-paraformaldehyde 8%

**Miguel Sanchez**

## Abstract

-I prepare 8% PFA (w/v) because I like to fix cells without even removing medium (because of the microtubules). If you want to use it 4% you can just dilute 1:1 with fresh PBS1X.

- Usually prepare about 250ml batches each time so you'd need ~20g of paraformaldehyde. Both PFAs we have in the lab will work just fine; I prefer the posh one (TAAB pellets) because they are slightly higher grade and they do not form dust (very toxic). But the Sigma powder will work just fine as well. If you use Sigma powder, you should weigh it inside the fume hood too.

- I first dilute and rise the pH to solubilize just in water. You may add a shorter volume (~150ml) to rinse later, after adding the pH correcting volumes. Remember that you'll also need a 10% volume (25ml in this example) of PBS10X at the end of the protocol!. Of course use mQ water. The mix will be very milky and acid at the start. Prepare in a suitable glass flask, I usually use a 2X volume Erlenmeyer.

- Start mixing and heating moderately inside the fume hood. You may put a parafilm "cap" to minimise fumes and evaporation. Overheating (>55degrees) is to be avoided, use a thermometer if you don't trust your hand. If you play properly with pH, you won't have any trouble and won't need much heating, not even when using the pellets.

- Add NaOH (I would recommend high concentrations, 10N) drop wise. Probably you'll need to add about 2mls until the soution clears. It usually takes about 20-30min. so be patient and wait for a while every time you add a small 250microl volume or so.

- Once cleared, you can adjust the pH back from the very high (>10) you've got now, to what it should be (7-8), using HCl. Usually you need about 1ml of 1M HCl.

- Once adjusted, you can rinse the total volume adding your 10% V of PBS10X and H2O.

- Let cool before aliquoting and freezing. I usually freeze it as it is, and then always, before use upon thawing, I filter it with a .45micron. It's very normal after readjusting pH to have some tiny "fibbers" or aggregates; they won't change upon removal the effect of your fixation, but they will be a source of a lot of trouble (very bright autofluorescence, pH goes acid if they stay) if you don't filter.

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## Protocol