Old-school (field standard) fix, digest, permeabilization for immuno or FISH applications

Buffer B - 1.2M sorbitol, 0.1M potassium phosphate buffer, 7.5 pH.

- Grow cells in growth media.
- Mix cells in the growth media with formalin (~37% formaldehyde; fresher is better) to make the final solution about 10% formalin.
- Let sit at RT for 1 hour. Invert a few times.
- Spin down the solution at < 3000g for however long it takes.
- Aspirate the fixative solution, spinning more if necessary. If you use a 50ml falcon, you’re going to have to be very careful to actually get a pellet, and may have to: aspirate all but 10ml, vortex, spin again. With higher cell densities a 14ml falcon is much better. A swinging bucket is always better!
- R/S and transfer to an eppendorf, using 1ml of Buffer B.
- Spin, aspirate, R/S Buffer B.
- Spin, aspirate, R/S Buffer B.
- Spin, aspirate, R/S in spheroplasting buffer (per 1ml: 898ul Buffer B + 100ul 200mM vanadyl ribonucleoside complex (NEB) + 2ul Beta-mercaptoethanol + 100U of lyticase (Sigma)).
- Incubate at 37C until cells are mostly (50%-90%, ???) phase-dark, but not too phase-dark. They should look darker under a phase contrast microscope than undigested cells, but not fragmented and falling apart. Try some negative and positive (overdigested) cells, record your impressions of their phase-darkness, and compare to later results to see what is optimal.
- Put on wet ice for 1min to stop the reaction.
- Spin 1200g for 3min (4C or RT works).
- Aspirate the clear supernatant. There should be a large fluffy pellet. R/S 1ml cold Buffer B.
- Aspirate the clear supernatant. There should be a large fluffy pellet. R/S 1ml cold Buffer B.
- Aspirate the clear supernatant. There should be a large fluffy pellet. R/S 1ml cold Buffer B.
- Aspirate the clear supernatant. There should be a large fluffy pellet. R/S 1ml 70% ethanol, put at 4C.