Gluteraldehyde-Paraformaldehyde Fixation

Protocol
1. While cells are growing prepare fixative. The gluteraldehyde concentration needs to be optimized for each protein of interest, however adding **6.25 ul of 8%** gluteraldehyde per ml of 16% paraformaldehyde is typical. Store on ice.
2. Aliquot 20µl of 1M NaPO4 pH 7.4 into 1.5 ml microfuge tubes and label.
3. Immediately prior to sampling add 100µl of fix into each prepared tube.
4. Take 500µl aliquots of each culture and add to appropriately labeled tube. Invert tubes 1 or 2 times.
5. Incubate 15 minutes at room temperature followed by 30 minutes on ice.
6. Pellet cells in microfuge and wash three times with 1 ml PBS. Resuspend pellets in GTE to a final OD of approximately A600=0.200.

NOTES:
1) If possible, grow cells in defined minimal medium for best results.
2) Spinning the cells to wash them after fixation can alter the localization pattern of proteins of interest. It is possible to wash cells by filtering them. See Lemon and Grossman (1998) Localization of Bacterial DNA Polymerase: Evidence for a Factory Model of Replication, Science 282: 15161519.

Solutions
16% paraformaldehyde
8% gluteraldehyde
PBS
GTE
1M NaPO4 pH 7.4

STORAGE

- Aliquots of Glutaraldehyde and paraformaldehyde are stable for 2 years in eppendorf tubes.

- Paraformaldehyde should be stored at room temperature to avoid precipitation (I have moved the aliquots, now they are next to the paraformaldehyde glass vials below the hood).

- Glutaraldehyde should be stored at 4 degrees. When making new aliquots of glutaraldehyde, make sure to label them with the date so that we can throw them out when they are 2 years old.