

Fixation for Preservation of GFP signals

- Mix culture 1:1 with 4% paraformaldehyde solution (2% final concentration) and incubate shaking at growth temperature for 20 minutes. Time can be adjusted for specific signals.
- Wash 2X PBS and proceed with DAPI or actin stain.
- For Hoechst stain use a 1/200 dilution of 1 mg/ml stock of dye and incubate for 30 minutes room temp (0.5 ml total volume) and then wash 2X with PBS.
- Resuspend in mounting medium.

Making paraformaldehyde

It is important to use solution that was made fresh on the same day or has been stored at -20 C. Avoid repeated freezing and thawing so store in aliquots.

- Dissolve 4g paraformaldehyde in 50 ml H₂O and add 1 ml 1M NaOH
- Stir at 65 C until dissolved. (try to do in a hood or cover to avoid exposure to fumes)
- Add 10 mls 10X PBS and allow to cool.
- Adjust pH to 7.4 using 1M HCl (need approximately 1 ml).
- Adjust final volume to 100ml with H₂O.

Mounting medium

Dissolve 100mg p-phenylenediamine in 10ml PBS and bring to the volume of 100ml with glycerol. Mix thoroughly and store at -70°C.