Fluorescence mounting medium (Antifade):

(from Spector Lab http://spectorlab.labsites.cshl.edu/protocols/#toggle-id-2)

Materials Needed

- 20ml glass scintillation vial
- Small stir bar
- Foil
- Glycerol
- 1X PBS
- Pipets
- * P-phenylenediamine (EMD Chemicals Inc. Cat# PX0730)
- Carbonate-Bicarbonate Buffer (see below)

1. Wrap a glass scintillation vial with foil and drop in a small stir bar. (PPD is light-sensitive.)
2. With a 10 ml pipet add 9 ml of glycerol to the vial.
3. With the 1000 ml Pipetman add 1ml of 1X PBS.
4. Place on stirrer and begin mixing.
5. Weigh out 10 mg of p-phenylenediamine on the Mettler balance. *PPD is toxic. Wear gloves and don't inhale it.*
6. Add the PPD to the vial and stir until it is all in solution (1-2 hrs.). The medium should appear almost colorless to a slight tint of yellow. If it is an intense yellow or orange color the PPD is most likely contaminated and will have background staining.
7. pH the mounting medium to a pH of 8.0-9.0 using the Carbonate-Bicarbonate buffer. pH paper of range 6.5-10.0 should be used to check the pH of the medium after addition of 12 drops of the Carb-Bicarb buffer and stirring. Additional drops of buffer are added until the desired pH is reached.
8. Aliquot the mounting medium and store at -70ºC.

* Flakes of PPD are large and should be crushed

Carbonate- Bicarbonate Buffer

1. Make up a 0.2M solution of anhydrous sodium carbonate (2.12g/100ml)
2. Make up a0.2M solution of sodium bicarbonate (1.68g/100ml)
3. Take 4 ml of A + 46 ml of B and bring up to 200 ml with DH2O. The pH will be 9.2.

*Note: If the PPD is contaminated or goes bad (turns a brown color) it will stain DNA, so each preparation should be tested. Check by looking at mitotic cells to be sure that chromosomes are not stained.