Mounting Medium

Mounting media can be prepared in the lab or commercially available ones can be purchased from several of the companies dealing in fluorescent probes.

Mounting medium can be made with 9 parts of glycerol and 1 part PBS. The pH should be adjusted to between 8.5 and 9.0. This pH has been found to be optimal by many investigators in preventing fluorescein and rhodamine quenching. pH's above and below this range will lose fluorescence much more quickly.

A small amount of an antiquench agent or free radical scavenger may also be added to the mounting medium as an added precaution. Some of these are:

1. p-phenylenediamine
2. propyl gallate
3. 1,4-Diazabicyclo (2,2,2)-octane (DABCO)
4. Ascorbic acid (That's right folks, vitamin C!)

Buffered glycerol with anti-fade.

Buffered glycerol is used mainly for fluorescent immunohistochemical preparations. The high pH provides for optimally efficient fluorescence of the commonly used labels. The added *p*-phenylenediamine (PPD) (Platt & Michael, 1983) or *n*-propyl gallate (Longin et al., 1993; Battaglia et al., 1994), retards fading.

Buffer:

Either 0.1M Phosphate buffer (pH 7.4): 10 ml *or* 0.1M TRIS buffer (pH 9.0): 10 ml

Anti-fading agent:

Either p-Phenylenediamine hydrochloride: 100 mg or *n*-propyl gallate: 500 mg

Glycerol: 90 ml

Keeps for at least 3 months, probably much longer, in darkness (which protects the anti-fade agent) at -20C. The working bottle is kept at 4C, for a week or two.

This does not solidify, but the coverslip can be held in position by applying a little nail varnish to its edges.