Homemade Antifade Solution

Mounting media is cheap and easy to make yourself, and gives better results than many of the expensive commercial options out there. You only need three things from a fluorescence mounting media…it should buffer your sample and fluorophores, prevent photobleaching, and have a high refractive index.

- 20mM (final concentration) Tris, pH 8.0. Many fluorophores are brighter at higher pH.
- 0.5% N-propyl gallate. This stuff helps prevent photobleaching.
- 50-90% Glycerol. Glycerol raises the refractive index of the mounting media, so you'll get brighter and higher resolution images. The higher the glycerol concentration, the better the fluorescence image but the worse the DIC (Nomarski) image. If you are doing fluorescence only, use 90% glycerol (yes, it's a pain to pipette – we promise it's worth it!). If you need to take a DIC image as well, use 50% glycerol so you don't lose the DIC contrast.

Recipe:

- Prepare a stock solution of 20%(w/v) n-propyl gallate (Sigma 02370) in dimethyl formamide
  n-propyl gallate 2g
  dimethyl formamide 10ml
  Note: n-propyl gallate does not dissolve well in water-based solutions

- Thoroughly mix
  1M Tris, pH8.0 2ml
  H2O 8ml
  Glycerol 90ml
  slowly add 2.5ml 20% n-propyl gallate dropwise with rapid stirring

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.

Bonus:

Glycerol jelly (For holding the embryos in place)

This traditional mounting medium is the most difficult one to use. If you can make a decent preparation in glycerol jelly, you'll be able to use any other aqueous mountant with your eyes shut.

Gelatin powder 10 g
Water 60 ml
Dissolve by warming and add
Glycerol 70 ml
Add *either* one drop of saturated aqueous solution of phenol ("liquid phenol") *or* 15 mg of sodium merthiolate as an antibacterial agent. This can be kept for a few weeks at 4C. Discard when it becomes turbid or moldy.

Glycerol jelly must be must be warmed to about 40C to melt the gel before using. It commonly needs to be freed of air bubbles, too. This can be done by warming the bottle (with cap loosened) in a vacuum-embedding chamber.

After coverslipping with glycerol jelly, leave the slide on a warm (40-45C), flat surface for about 30 minutes, to let the medium soak into the section, and then remove it to a cool place to set. It is quite difficult to make a bubble-free preparation with this medium, and the bubbles can be tiny and numerous. Check with a microscope while the slide is still warm. If it's no good, remove the coverslip by soaking in warm water, and try again.

This medium has a low refractive index (1.42). Because of this, many unstained structures remain visible, which may be an advantage or a disadvantage, depending on what you expect to see in the finished preparation.