

Application Letter 4
Bleaching Protection and Mounting Media

BLEACHING PROTECTION**P-phenylenediamine or PPD**

(Sigma Chemical Co.) appears to be the best anti-fade agent. It works well with fluorescein but has a peculiar effect on DAPI, in which it induces a dim red fluorescence. It is used at a concentration of 0,1% in a mountant containing 10% phosphate-buffered saline and 90% glycerol. This mountant should be stored at -20 C or better , -70 C. It goes brown but can still be used until dark brown.

Attention:

this agent has carcinogenic properties and should be handled with great care.

Diazabicyclo-octane or DABCO

(Aldrich # D2, 780-2) is less effective as the above mentioned PPD, but has the advantage of lower toxicity and easier storage, being stable at room temperature.
It is generally also used in a concentration of 0.1%.

MOUNTING MEDIA

Mowiol can be used with the addition of PPD or DABCO as antifade agent. The following procedure was recommended by C. Broesamle, Brain Research Institute, University of Zurich.

6 g Glycerol
2.4g Mowiol 4-88 (Calbiochem # 475904)
6 ml ddH₂O

Add 12 ml 0.2 M Tris buffer pH 8.5 and mix for about half a day on a shaker.

Let the mixture sit for 2 hours

(the Mowiol will not dissolve completely).

Incubate at 50 degrees centigrade for 10 min.

centrifuge mixture at 5000g for 15 min.

Aliquot and freeze at -20 C until use.

Note:

When thawing, make sure that the Mowiol solution has room temperature before mounting specimen. Otherwise many small air bubbles will form under the coverslip later.

Gelvatol is the trade name of a form of polyvinyl alcohol that sets solid after drying. The original product may be hard to find, but an equivalent material is **Airvol 205** (Air Products Nederland B.V., P.O.Box 85075-3508 AB Utrecht.

The following recipe was recommended by M. de Brabander (see Virology 12.316-317 (1960).

Dissolve 20 g Gelvatol in 80 ml of 0.14 M NaCl solution buffered with 0.01 M KH₂PO₄-Na₂HPO₄ at pH 7.2 and stir for 16h. Add 40 ml glycerol and stir for another 16h. Centrifuge at 12000 rpm to get rid of undissolved particles. Decant supernatant and check pH (between 6 and 7). Store in an airtight bottle and add 100 mg/ml DABCO before use to reduce fading.

Vectrashild seems to have even stronger anti-fade properties as Mowiol/PPD We quote Dr. J. Drazba (Director, Confocal Microscopy Facility), Department of Neurosciences, Cleveland Clinic Foundation, Cleveland, OH (1995): I used to us Mowiol with the addition of PPD as an anti-fade agent. I then discovered Vectrashield, from Vector Co. and liked it much better. We' ve done side by side tests with Vectrashield and other commercial mounting mediums as well as our home made mowiol/PPD and the Vectrashield prevented fading much longer. It is also equally good at preventing fading of both Fluorescein and Texas-Red. I've kept specimen in Vectrashield ar -20 C for over a year and see no degradation.

Canada balsam and certain epoxy resins

fluoresce strongly. This undesired effect can be used to its advantage in nonfluorescent specimen like mineral grains and siliceous shells as negative contrast detection.

Fluid mounts

like glycerol based media that do not harden, should be sealed with nail varnish in order to fix the coverslip permanently.

For any further information on this topic, we highly recommend R. Bacaloo et al., 1995 Guiding principles of specimen preservation for confocal fluorescence microscopy In: Handbook of biological confocal microscopy, second edition, Plenum Press