Application Sheet for Citifluor™ AF1 Mountant Solution

Usage

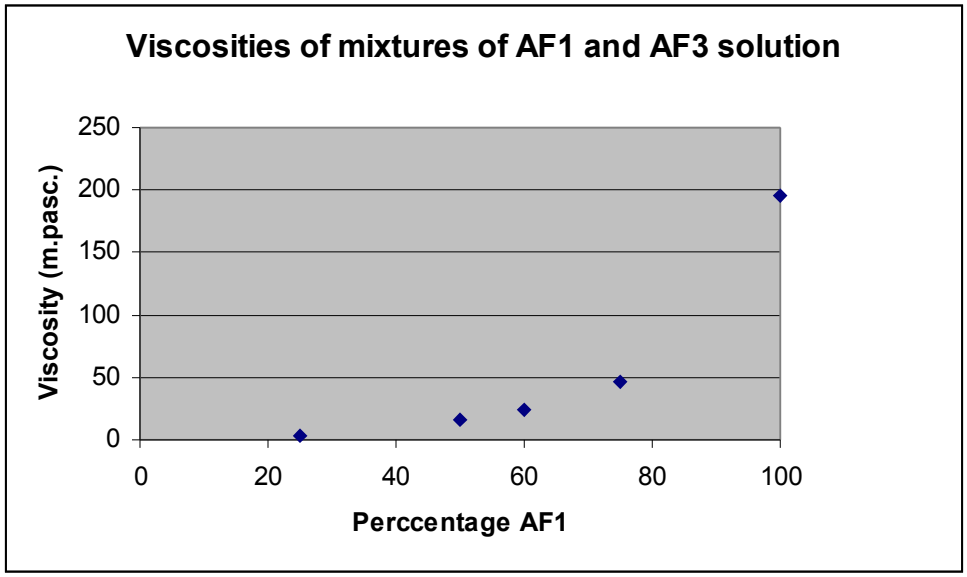
AF1 is a mountant solution composed of glycerol, phosphate buffered saline and an antifadent. It was specifically designed to stop the photobleaching of the fluorescein moiety of FITC labelled biological specimens. AF1 is useful for many other fluorochromes such as DAPI, rhodamines, Hoechst, Alexa and cyanine (Cy-3 and Cy-5) dyes, Texas Red, phycoerythrins and Green Fluorescent Protein(GFP). It is ideal for examining tissue sections and dead cells. In addition, it has been found useful for stabilising the AUTOFLUORESCENCE of species such as cyanobacteria.
AF1 solutions have been employed with the following techniques; fluorescence *in situ* hybridisation (FISH including CARD-FISH and confocal laser scanning microscopy (CLSM) are being used.
The solution has a pH of ~10. The solution should be pipetted onto the specimen and then a cover slip applied. If the slides are stored in a refrigerator, the viscosity of the mountant solution increases thereby helping too keep the cover slip in place. There is no need to seal the cover slip with nail varnish. Specimens mounted in AF1 solutions have been kept in this way for many months without suffering damage.

Properties and storage of AF1 mountant solution

The solution is of medium viscosity and has a water-white in appearance. It may be stored at room temperature and ideally between 5^0 and 15^0 and out of strong sunlight. The cap of the bottle or if using the pipette supplied with the material, the cap which covers the pipette delivery point, should always be replaced after use since the solution is hygroscopic. Samples stored under these conditions for 6 months have shown no apparent deterioration. If the AF1 solution is being used in an assay, a control experiment should always be carried out.

Obtaining the correct viscosity for your application

If the viscosity of the AF1 solution is too high for your purposes, it may be admixed with AF3 mountant solution. As the amount of AF 3 solution is increased so the viscosity decreases. Conversely, if you wish to have a higher viscosity add AF2 mountant solution to the AF1 solution. Increasing the amount of AF2 solution increases the viscosity.



Graph showing how the viscosity of AF1 solution is influenced by adding AF3 solution

Other useful advice:

(a) If there is some initial quenching of fluorescence.

In some cases, the reduction in the rate of PHOTOBLEACHING may be accompanied by a *reduction in the initial intensity of the fluorescence signal*. By diluting the AF1 solution with glycerol the reduction in the intensity of the fluorescence signal can oftentimes be mitigated.

Dilution of AF1 with glycerol will increase its refractive index whereas dilution with water will reduce its refractive index.

(b) Use as a hardening mountant.

To a poly(vinyl alcohol) e.g. Airvol 203(Air products) or Mowiol 4-88 (Calbiochem) solution (20% in water) add ~20% by volume of AF1. This solution is best used soon after it is prepared as it doesn't have a long shelf-life