

# Technical Data Sheet

## Citifluor Hardening Antifadents

Catalog #17977, 17978

Glycerol Based	High Refractive Index	Glycerol Free	High Refractive Index
Tris MWL 4-88 + AF200		PVP + Antifadent	Permafix PF/HRF
Tris MWL 4-88 + AF300		CVPOH + Antifadent	
CFPVOH + AF200		CFPVOH + AF100	
CFPVOH + AF300		Permafix PF + AF100	

### PVP plus Antifadent

Aqueous poly (vinyl pyrrolidone) is very useful as a permanent mountant that possesses some antifadent properties. The dry films of the polymer have a high refractive index.

The solutions are of relatively low viscosity and have a slight yellow color. They have a pH of ~9.5.

A few drops of the solution should be applied to the specimen followed by a cover slip. The water evaporates to give a clear film, which holds the cover slip in place, thereby aiding the safe storage of the specimen.

The samples may be stored at room temperature and are very stable, e.g. samples many years old have shown no sign of gelation.

### CFPVOH plus Antifadent

This is an aqueous solution of a special grade of poly (vinyl alcohol) containing an antifadent.

The solution is of medium viscosity and has a pH of ~8.5 and contains a small amount of sodium azide to prevent microbial growth.

A few drops of the mountant (~25 µl) should be applied to the specimen followed by a coverslip. If the edges of the coverslip are not sealed, water slowly evaporates to give a stable film that holds the coverslip in place.

The cap of the bottle should always be replaced after use to prevent contamination and evaporation of water. Samples of this solution have been kept for 6 months at room temperature without the appearance of any gel. Storage at low temperatures is not recommended.

### Single Pack High Refractive Index

#### Permafix PF/HRF

This is an aqueous solution of a chemically modified poly (vinyl alcohol) plus additives.

The solution is of medium viscosity and has a pH of ~7. If an antifadent is required, it is recommended that a small amount of the PF/HRF is admixed with AF100 (~10% v/v).

Permafix PF/HRF should only be used with specimens where the fluorochrome is covalently bound to the biological substrate. Intercalated fluorochromes may leach out of the substrate due to the formulation containing a surfactant-like species. The mountant has found use with the technique of CLSM where obtaining an accurate cell count was of the essence (106).

#### CFPVOH plus AF100

The CFPVOH is an aqueous solution of poly (vinyl alcohol) and the AF100 is a phosphate-buffered saline solution of an amine-based antifadent.

Solutions should be made up by mixing 1 part by volume of AF100 with 9 parts by volume of CFPVOH. These solutions should be used within 10 hours as the efficacy of the antifadent reduces with time. With this in mind, it is better to make up the mixture prior to use and not to rely on keeping solutions.

A few drops of the solution containing both components should be applied to the specimen followed by a cover slip. Evaporation of the water under these circumstances takes a few hours leaving a clear film, which holds the coverslip in place.

The combination of CFPVOH with AF100 has been used for examining specimens using CLSM (105, 106) and for specimens generated using FISH (109, 110). Fluorochromes such as DAPI (108, 110), Cy3, Cy5 (110), Alexa (107, 111, 112) and Hoechst dyes (105), and FITC (108, 110, 113).

### **Permafrix PF plus AF100**

The Permafrix PF is an aqueous solution of a chemically modified poly (vinyl alcohol). The addition of AF100 (ratio of Permafrix PF to AF100 being 10:1) leads to a catalyzed reaction producing a clear hard film which holds the coverslip firmly in place. It is recommended that a only small volumes of the mixture are made up since they tend to gel quickly (within a few hours).

A few drops of the solution containing both components should be applied to the specimen followed by a cover slip. Evaporation of the water under these circumstances takes a few hours leaving a clear film, which holds the coverslip in place.

The Permafrix solutions are of medium viscosity and contain a small amount of sodium azide to prevent fungal growth.

The caps on the bottles of the reagents should always be replaced after use and the solutions stored at room temperature (10° to 200°C).

### **Glycerol Containing Hardening Systems**

These are systems with the antifadents being contained in the AF200 and AF300 solutions. These antifadents may be used with the aqueous polymer systems MWL 4-88, CFPVOH and Permafrix PF.

#### **AF200**

This is a glycerol solution containing a non-basic antifadent. It has a relatively high viscosity. It is designed for use with specimens labeled with DAPI, Cy and Hoechst dyes.

Mixtures of AF200 and the polymer solutions should be made up on demand since the mixtures gel upon storage for long periods. Solutions of AF200 and the polymer solutions should be made up in the ratio of 1 part by volume AF200 to 3 parts per volume of the polymer solution.

#### **AF300**

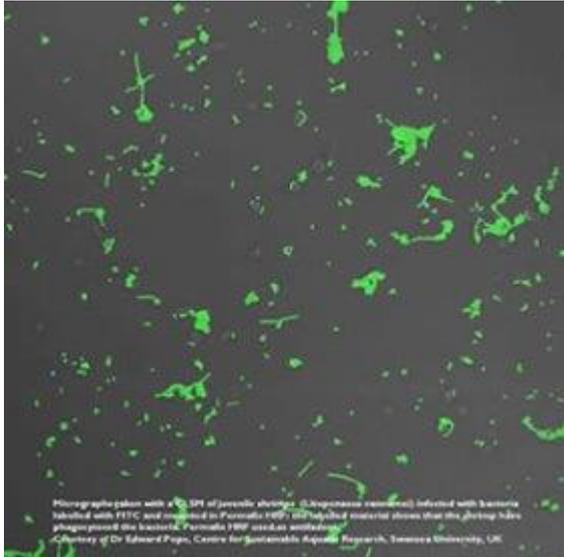
This is a glycerol solution of an amine-based antifadent and has a relatively high viscosity. It may be used with a wide range of fluorochromes.

Mixtures of AF300 and the polymer solutions should be made up on demand since the mixtures gel upon storage for long periods. Solutions of AF300 and the polymer solutions should be made up in the ratio of 1 part by volume AF300 to 3 parts per volume of the polymer solution.

#### **Tris-MWL 4-88**

This is a classical, popular mountant based on proprietary poly (vinyl alcohol), Mowiol® 4-88. The composition of the solution is well tried and tested and contains the polymer, glycerol and tris-amine buffer. It has a pH of ~8.5. It may be used as a hardening mountant on its own, but if antifadent properties are required, it should be used with either AF200 or AF300.

Solutions of MWL 4-88 should be stored at room temperature in a tightly sealed bottle.



*Permafrix HRF Example*

### CFPVOH

The CFPVOH mountant is an aqueous solution of poly (vinyl alcohol) containing a small amount of sodium azide to prevent microbial growth, and may be used with either AF200 or AF300 with which it is freely miscible. Solutions of the two reagents have a limited shelf life.

### Permafrix PF

Permafrix PF is an aqueous solution of a chemically modified poly (vinyl alcohol) and may be used with either AF200 or AF300 with which it is freely miscible. Solutions of the two reagents have a limited shelf life.