

EDITION 2

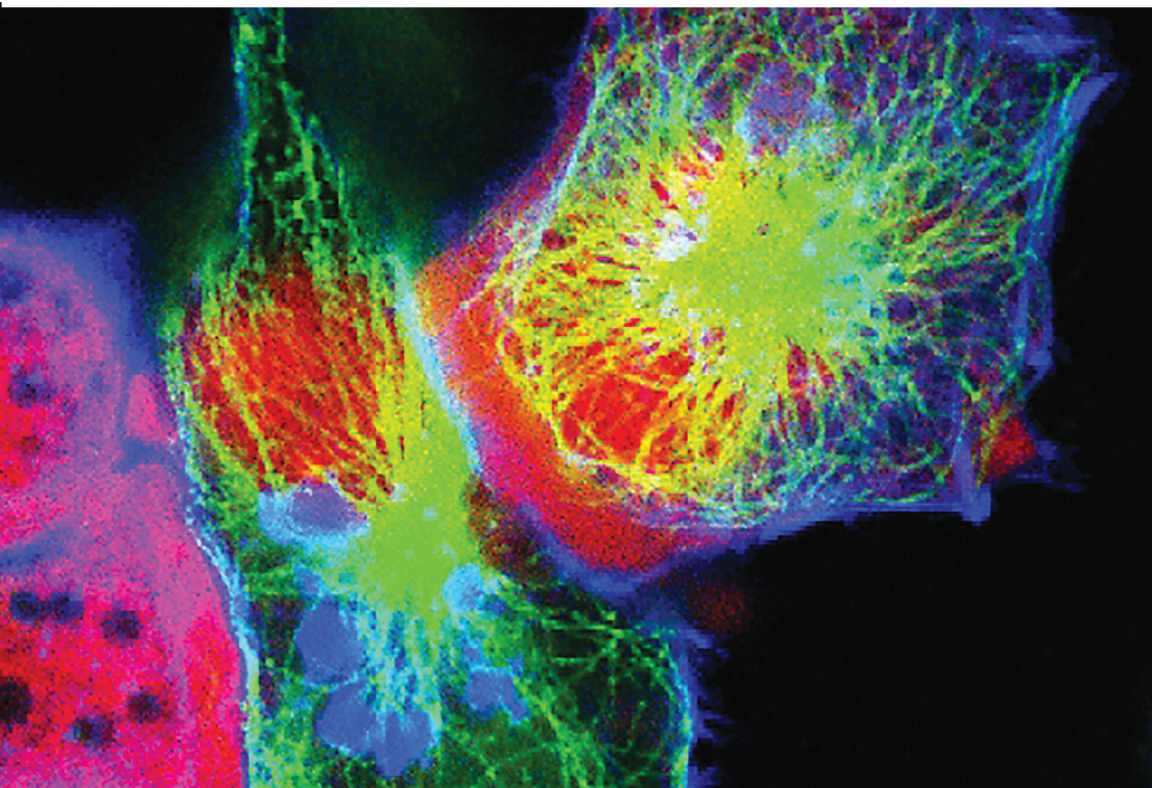
**citifluor**

A Division of EMS Acquisition Corp.

Electron Microscopy Sciences

Antifadent  
Mountant  
Solutions

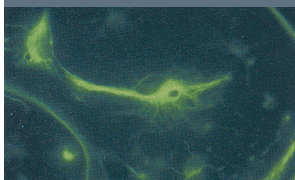
## The Antidote for Photobleaching



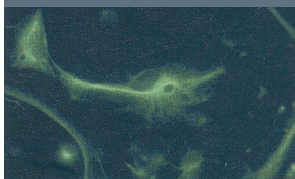
[www.citifluor.com](http://www.citifluor.com)  
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**Electron  
Microscopy  
Sciences**

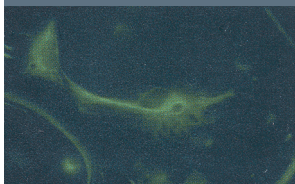
After 15 Seconds Illumination



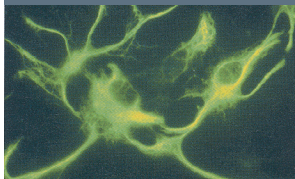
After 1 Minute Illumination



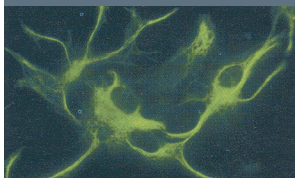
After 3 Minutes Illumination



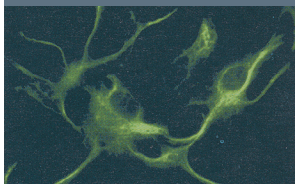
After 15 Seconds Illumination



After 5 Minutes Illumination



After 45 Minutes Illumination



## Citifluor Antifadent Mountant Solutions

### Mountant Media to Minimize Photobleaching

Antifadent Solutions reduce the photo-bleaching, or fading of the fluorescence of dyes used for labeling biological species. The fading of fluorochrome dyes is a particular problem in fluorescence microscopy, such as in immunofluorescence studies. Fluorescent dyes are also used as cell markers, for following the uptake and release of calcium from cells for example, and for characterizing cell surfaces. For work at high magnification, a non-fluorescent immersion oil is available.

In fluorescence microscopy, the fluorescence is stimulated by high intensity UV or visible light. Absorption of the light populates an excited state of the dye (usually a singlet state) and this leads to fluorescence. However, the excited state of the dye may undergo chemical reactions which leads to its destruction as evidenced by the fading or bleaching of the fluorescence and consequent loss of the image or in the case of assays, a change in signal intensity during measurement.

To overcome these problems, use antifadent (antibleaching) solutions as mounting media when specimens are examined by fluorescent microscopy or other detection systems and as additives for assays.

The most frequently used solution is AF1 which is an anti-fadent (anti-bleaching agent) contained in a glycerol PBS (phosphate-buffered saline) solution and is particularly useful for examining tissue sections.

AF2 solution contains the antifadent in glycerol which enables users to choose their own buffer, and AF3 is the antifadent in PBS solution. AF3 is particularly useful for examining live cells.

AF87 is a non fluorescent immersion oil containing antifadent. CFPVOH is an aqueous solution of poly (vinyl alcohol) for use as a solid mountant. AF100 is a solution of antifadents (anti bleaching agents) for use with CFPVOH when fading (bleaching) of specimens in a solid mountant is a problem.

None of the antifadents is based on p-phenylenediamine. All solutions may be stored at room temperature.

### Applications

Citifluor mountant media are proving of value in many areas of the Biological Sciences such as Plant Sciences, Limnology, Bacteriology, Histology, Immunochemistry, Neurology and Oceanography.

They are used in many analysis techniques such as immunofluorescence, epifluorescence microscopy, confocal laser scanning microscopy (CLSM) and total internal reflection microscopy (TIRF), as well as fluorescence hybridization (FISH) and Catalyzed Reporter Deposition coupled with FISH (CARD FISH).

### Quality and Availability

We have been producing high quality antifadent solutions for minimizing Photobleaching both as hardening and non hardening mountants, for over 25 years for many clients world-wide: hospitals, research institutes, path labs, universities, medical centers and forensic labs. All our antifadent solutions/photobleaching solutions products are regularly tested and found to be of consistent high quality and mostly out-perform other commercial products in their anti-bleaching capacity. They are safe to handle, being of very low toxicity and non-irritant.

These are the critical decision factors which will help you select the most appropriate mountant:

*Do you need a Hardening or a Non Hardening medium?*

*If your system is Glycerol tolerant than choose a Glycerol-Based product, if not then use a Glycerol-Free solution.*

These high refractive index mountant solutions are to be used where you wish to obviate the effects of spherical aberration (caused by a mismatch between the refractive index of the glass of the coverslip and the mountant medium) which leads to a loss of resolution of your images.

The table below illustrates the main types of antifadent available.

	Glycerol-Based Mountants		Glycerol-Free Mountants	
Non-Hardening Mountants	<b>AF1</b> <b>AF1 Plus DAPI</b> <b>AF2</b> <b>AF4</b> <b>AFP1</b>	<b>HIGH REFRACTIVE INDEX</b> <b>CFM-1</b> <b>CFM-2</b> <b>CFMR-2</b> <b>CFM-3</b> <b>CFM-1 Plus AF</b>	<b>CFPVOH Plus AF100</b> <b>AF3</b> <b>AFR3</b>	<b>HIGH REFRACTIVE INDEX</b> <b>AF87</b>
Hardening Mountants	<b>Tris MWL 4-88 + AF200</b> <b>Tris MWL 4-88 + AF300</b> <b>CFPVOH + AF200</b> <b>CFPVOH + AF300</b>		<b>PVP + Antifadent</b> <b>CFPVOH + Antifadent</b> <b>CFPVOH Plus AF100</b>	

If you are unsure about which mountant to use, please don't hesitate to contact us, either by phone or email. We have technical staff on hand who are happy to answer the simplest, or the most challenging of questions.

Many of our products are designed for routine applications where consistent quality and low cost are essentials. The fact that many pathology laboratories place standing orders for large volumes is testimony to the quality and the "value for money" afforded by these products. For users ordering AF1 solution in bulk the cost is much lower. No other commercially available antifadent matches this price thus making us undoubtedly the most cost effective supplier of anti-bleaching agents.

**All our solutions and reagents are prepared by a qualified (Chartered) chemist.**

**All our solutions are prepared in the US.**

**Our High Refractive Index mountants are unique. There are no other commercially available mountant solutions having a refractive index which match glass and enable visualization at such depths within a sample.**



### Glycerol Based

- **AF1** A glycerol-PBS solution contains an amine antifadent.
- **AF1 plus DAPI** contains both an amine antifadent and the DNA stain, DAPI.
- **AF2** A glycerol solution contains an amine antifadent that allows you to choose your own buffer solution.
- **AF4** A glycerol solution of n-propyl gallate.

### High Refractive Index Glycerol Based Solutions

These solutions have a refractive index that matches that of the glass of the coverslip thereby minimizing the effects of spherical aberration. Such mountants are invaluable for imaging the internal structures of samples by techniques such as single multi-photon confocal niche since it acts as an efficient Clearing Solution (clearing occurs within a few minutes – Sean Speese, Oregon Health and State University, Personal Communication). The beneficial effects of these reagents can be seen in the two figures below (see page 10) where it can be seen that high resolution images have been obtained at a significant depth within the mountant solution. Additional features of these solutions include their total water miscibility, they are non-odorous and do not quench the fluorescence of fluorochromes (unlike thiodiethanol, another proposed high refractive index mountant) and the solutions are stable for long periods (at least 1 year).

- **CFM-1** is a glycerol-PBS buffered solution having a refractive index of ~1.52 which can be used for transmission microscopy as well as with epifluorescence microscopy.
- **CFM-1 plus AF** is a glycerol-based solution having a refractive index of ~1.52 (at room temperature) that contains an amine antifadent

- **CFM-2** is a glycerol-tris amine buffered solution having a refractive index of ~1.52 (at room temperature) and a pH of ~8.5
- **CFM-3** – This glycerol-based antifadent contains a phenolic antifadent of neutral pH and a refractive index of ~1.52 which also acts as a Clearing Solution enabling visualization of fluorochromes deep within the sample.
- **CFMR2** – This product was designed for use with samples labeled with GFP. It contains a unique antifadent which does not de-oxygenate the solution which is an essential property if the GFP is not to bleach.

### Glycerol Free

- **AF3** is a PBS solution containing an amine-based antifadent.
- **CFPVOH** is an aqueous solution of poly (vinyl alcohol). Provided care is taken not to allow the water to evaporate, it will remain fluid. Retardation of photobleaching is effected by the addition of the aqueous antifadent solution AF100.
- **AF100** is a PBS solution of an amine-based antifadent that if used as an additive to CFPVOH (1 part AF100 to 9 parts CFPVOH). Provided care is taken not to allow the water to evaporate, it will remain fluid.
- **AFR3** is a PBS solution containing a NEW non-amine, non phenolic antifadent.

### High Refractive Index Glycerol Free Solutions

- **AF87** is an immersion oil having a refractive index of 1.52 and contains an antifadent. It may be used as an immersion oil and also as a mountant. Since AF87 is an oil that is immiscible with water, it is essential that specimens are dehydrated before application of the mountant.

### Citifluor Non-Hardening Antifadents

Glycerol Based	High Refractive Index	Glycerol Free	High Refractive Index
AF1	CFM-1	CFPVOH + AF100	AF87
AF1 + DAPI	CFM1 + AF	AF3	
AF2	CFM-2	AFR3	
	CFMR2		
AF4	CFM-3		

### Glycerol-Based Anatidae Reagents

	pH	RI	P/G*
AF1	~9	1.463	P
AF2	NA	1.473	P
AF4	NA	1.476	G

\* P = HDPE plastic bottle, G = Brown glass bottle

\*\* Please ask for a quotation for larger volumes

Hardening mountants are aqueous solutions containing a polymer such as poly (vinyl alcohol) (PVOH). When a few microliters of these solutions are pipetted onto a microscope slide and a coverslip applied, evaporation of water slowly takes place and the formation of a stable film results which immobilizes the coverslip.

A variety of hardening mountants is available which provide films possessing a range of hardness. They are based on water-soluble polymers such as poly (vinyl alcohol (PVOH) and poly (vinyl pyrrolidone) (PVP).

**Why are aqueous glycerol solutions of PVOH containing antifadents inherently unstable?**

PVOH is produced by hydrolysis of poly(vinyl acetate) and most commercial samples of PVOH contain residual (unhydrolyzed) acetate groups. These groups undergo hydrolysis, often accelerated by the added antifadents, during storage and this can cause a change in pH and more usually gelation.

This latter process leads to an unpredictable shelf-life. By making up small volumes of PVOH solution containing the required amount of antifadent solution, you have materials of consistent composition and performance as well as making better use of your purchased materials.

**Glycerol Based**

- **AF200** is a glycerol solution containing an amine-based antifadent.
- **AF300** is a glycerol solution containing a phenolic type of antifadent.

**Film Forming Polymer Solutions**

- **Tris-MWL 4-88** is a classical, popular mountant solution based on Mowiol® 4-88, glycerol, water and tris-amine buffer. Following evaporation of the water a film of weak to medium strength is formed. To have effective reduction in

photobleaching, it should be used with either AF100, AF200 or AF300.

- **CFPVOH** is an aqueous solution of poly (vinyl alcohol) and is designed to be used with the glycerol-based antifadent solutions AF200 or AF300. Following evaporation of the water films of medium hardness are produced.

**Glycerol Free**

- **PVP plus antifadent** is an aqueous solution of poly (vinyl pyrrolidone) containing an amine-based antifadent. The solutions are stable over long periods e.g. in excess of five years.
- **CFPVOH plus antifadent** is an aqueous solution of a carefully selected PVOH containing an amine-based antifadent and has a shelf life 6 months.

**Film Forming Polymer Solutions**

- **CFPVOH** is an aqueous solution of poly (vinyl alcohol). Retardation of photobleaching is effected by the addition of the aqueous antifadent solution AF100. (1 part AF100 to 9 parts CFPVOH)

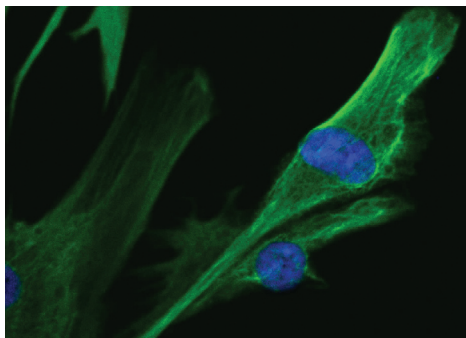
**Citifluor Hardening Antifadents**

**Glycerol Based**

- Tris MWL 4-88 + AF200
- Tris MWL 4-88 + AF300
- CFPVOH + AF200
- CFPVOH + AF300

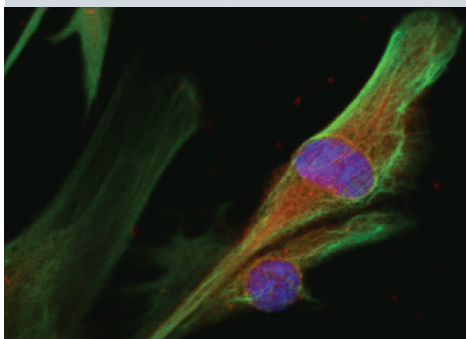
**Glycerol Free**

- PVP + Antifadent
- CVPOH + Antifadent
- CFPVOH + AF100



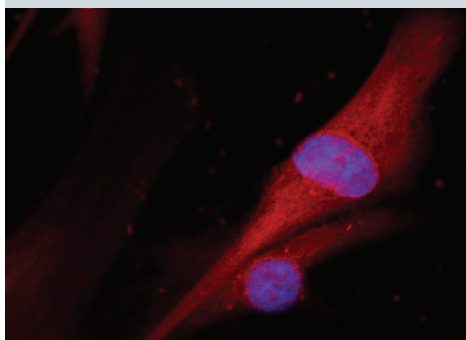
Mounted in Citifluor AF1. GFAP (glial fibrillary acidic protein) labeled with Alexa 568. Vimentin GFAP complex. Hoechst 33342 nuclear stain. Vimentin Alexa 488.

Courtesy of Kin Pong U and Dr. Patrizia Ferreti of the Institute for Child Health UCL UK.



Mounted in Citifluor AF1. GFAP (glial fibrillary acidic protein) labeled with Alexa 568. Vimentin GFAP complex. Hoechst 33342 nuclear stain. Vimentin Alexa 488.

Courtesy of Kin Pong U and Dr. Patrizia Ferreti of the Institute for Child Health UCL UK.



Mounted in Citifluor AF1. GFAP (glial fibrillary acidic protein) labeled with Alexa 568. Vimentin GFAP complex. Hoechst 33342 nuclear stain. Vimentin Alexa 488.

Courtesy of Kin Pong U and Dr. Patrizia Ferreti of the Institute for Child Health UCL UK.

## ■ Citifluor AF1 Mountant Solution

### Usage

AF1 is a mountant solution composed of glycerol, phosphate buffered saline and an antifade. It was specifically designed to stop the photobleaching of the fluorescein moiety of FITC labeled 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 stabilizing the AUTOFLUORESCENCE of species such as cyanobacteria. AF1 solutions have been employed with the following techniques; fluorescence in situ hybridization 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 to 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

The solution is of medium viscosity and has a water-white in appearance. It may be stored at room temperature and ideally between 50° and 150° 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 AF3 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.

### Citifluor AF1 Mountant Solution (continued)

**Right:** 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:

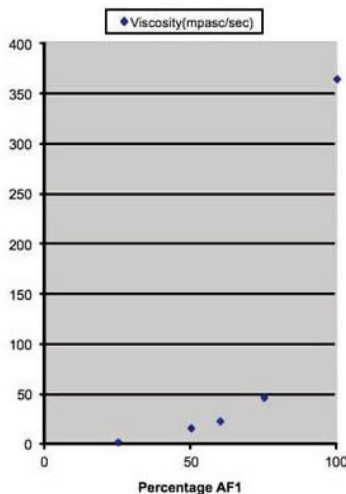
In some cases, the reduction in the rate of PHOTOBLEACHING may be accompanied by a reduction in the initial intensity of the fluorescence signal. By diluting the AF1 solution with glycerol the reduction in the intensity of the fluorescence signal can oftentimes be mitigated.

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.

	Cat No.	Description	Qty.
RT	17970-25	Citifluor AF1	25 ml
RT	17970-100	Citifluor AF1	100 ml



## ■ Citifluor AF1 plus DAPI Mountant Solution

### Usage

AF1 is a mountant solution composed of glycerol, phosphate buffered saline an antifadent to which DAPI has been added at a concentration of 2 µg per ml. If a lower concentration of DAPI is required, dilution should be carried out by adding a further quantity of AF1 solution. The solution will be of value to those using the FISH technique since the DAPI will stain the nuclei and the antifadent will prevent its bleaching.

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 to 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

The solution is of medium viscosity and has a water-white in appearance. It may be stored at room temperature and ideally between 50° and 150° 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.

	Cat No.	Description	Qty.
RT	17970-125	Citifluor AF1 + DAPI	15 ml

# Non-Hardening Mountant Solutions

## Glycerol-Based Solutions

### ■ Citifluor AF2 Mountant Solution

#### Usage

AF2 is a mountant solution composed of glycerol and an antifade and is optically transparent from 300nm into the 750nm. It was specifically designed to stop the photobleaching of the fluorescein moiety of FITC labeled biological specimens. Its application is not however limited to FITC labeled materials and has been used with advantage with many other fluorochromes including rhodamines, DAPI and GFP. It is ideal for examining tissue sections and dead cells. The AF2 mountant has been employed with the following techniques: fluorescence in situ hybridization (FISH), and confocal laser scanning microscopy (CLSM).

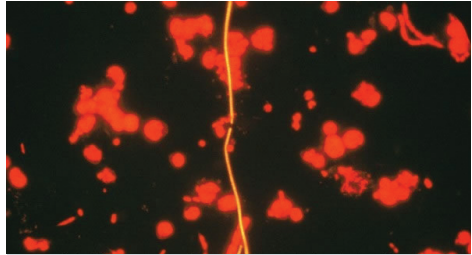
The AF2 mountant is ideal for examining tissue sections and dead cells. An aqueous solution (75% AF2 to 25% water v/v) has a pH of ~10. The mountant solution should be pipetted onto the specimen and then a cover slip applied. If the slides are stored in a refrigerator, the viscosity of the mountant solution increases thereby helping to keep the cover slip in place. There is no need to seal the cover slip with nail varnish. Specimens mounted in AF2 have been kept in this way for many months without suffering loss of fluorescence.

#### Properties and Storage

The solution is of medium viscosity and has a water-white in appearance. It may be stored at room temperature and ideally between 50° and 150° 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 in order to prevent the ingress of water (due to the glycerol being hygroscopic). Samples stored under these conditions for 6 months have shown no apparent deterioration. If the AF2 solution is being used in an assay, a control experiment should always be carried out.

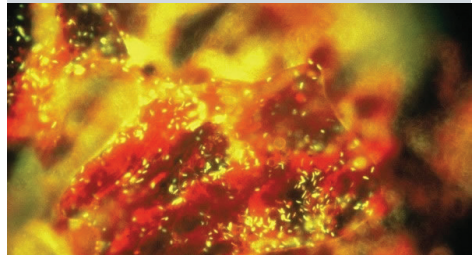
#### Obtaining the Correct Viscosity for Your Application

If the viscosity of the AF2 solution is too high for your purposes, it may be admixed with either AF1 or AF3 mountant solution. As the amount of AF1 or AF3 solution is increased so the viscosity decreases.



Autofluorescence of green algae and a bacterium from the Antarctic glacier mounted in AF2 solution.

Courtesy of Dr. Wynn-Williams, British Antarctic Survey.

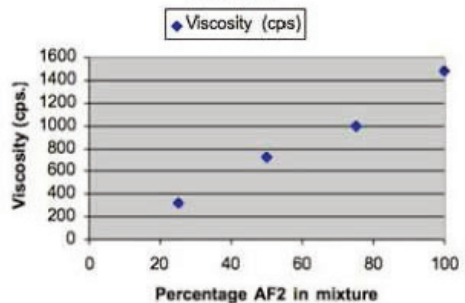


Antarctic bacteria on soil grain stained with Acridine Orange and mounted in AF2 solution.

Courtesy of Dr. Wynn-Williams, British Antarctic Survey.

**Below:** Graph showing how the viscosity of AF2 solution is influenced by adding AF1 solution

Effect upon the viscosity of AF2, of adding AF1 solution





### Citifluor AF2 Mountant Solution (continued)

#### Would You Like to use AF2 in a Hardening Mountant?

AF2 has also been used to create a hardening formulation by adding it (~20% by volume) to an aqueous solution of poly (vinyl alcohol), e.g. Airvol 203 or Mowiol® 4-88.

	Cat No.	Description	Qty.
<input type="checkbox"/> RT	<b>17971-25</b>	Citifluor AF2	25 ml
<input type="checkbox"/> RT	<b>17971-100</b>	Citifluor AF2	100 ml



## ■ Citifluor AF4 Mountant Solution

### Usage

AF4 is a mountant solution composed of glycerol and the antifade n-propyl gallate. N-Propyl gallate is a well established antifade and is particularly useful for stopping the photobleaching of DAPI, Hoechst and Alexa dye stained materials, as well as FITC labeled materials. It is ideal for examining tissue sections and dead cells. An aqueous solution (75% AF4 to 25% water v/v) has a pH of ~5. Since many fluorochromes exhibit their maximal fluorescent intensity at higher pH values the AF4 should be mixed with an appropriate buffer solution. The resulting solutions may start to show discoloration within a few hours and therefore buffered AF4 solutions should be made up as and when required. The mountant solution should be pipetted onto the specimen (which has been copiously washed with the appropriate buffer) and then a cover slip applied. If the slides are stored in a refrigerator, the viscosity of the mountant solution increases thereby helping to keep the cover slip in place. There is no need to seal the cover slip with nail varnish.

### Properties and Storage

The solution is of medium viscosity and has a water-white in appearance. It may be stored at room temperature and ideally between 50° and 150° 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 as a matter of good practice and also to prevent the ingress of water (due to the glycerol being hygroscopic). Samples stored under these conditions for 6 months have shown no apparent deterioration. If the AF4 solution is being used in an assay, a control experiment should always be carried out.

	Cat No.	Description	Qty.
<input type="checkbox"/> RT	<b>17973-25</b>	Citifluor AF4	25 ml



# Non-Hardening Mountant Solutions

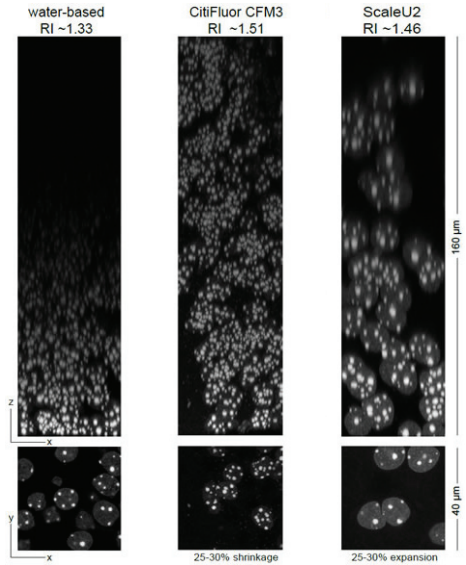
Glycerol-Based, High Refractive Index Solutions

## Reagents for Transmission Microscopy as well as Fluorescence Microscopy

All the following solutions have a refractive index of  $\sim 1.52$  at room temperature ( $20^{\circ}\text{C}$ ) and are freely miscible with water. They may be used as an immersion oil in the standard way as well as a mountant solution. The refractive index of the materials makes them ideal for use when specimens are being examined by confocal laser scanning microscopy (CLSM). They show very little absorption above 400nm.

The CFM range of mountant solutions should only be used with samples that have been fixed (4% paraformaldehyde in phosphate buffered saline for 30 minutes). The crosslinking (fixing) of the sample prevents non-covalently bonded fluorochromes, e.g. DAPI, Hoechst dyes, from becoming detached from the specimen etc.

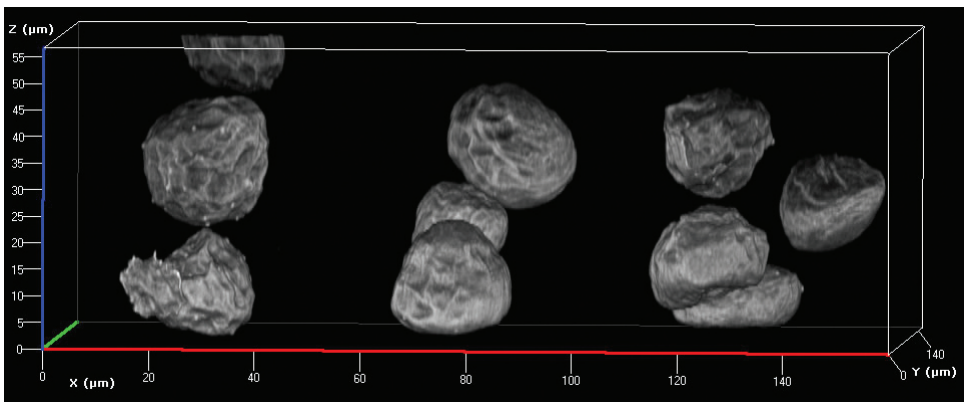
If some bleeding of the dye(s) is still observed a further fixation with paraformaldehyde should be carried out. For sealing the coverslips, when CFM mountant solutions are used, the product CoverGrip<sup>®</sup> marketed by Bioutium has been recommended. (We are grateful to Dr. Sean Speese, Jungers Center for Neuroscience, Oregon, U.S.A. for these many helpful suggestions).



The two images are examples of CitiFluor product applications, kindly provided by Dr. Sean Speese:

**Above:** 63x1.4 NA PlanApo/1 Airy/Nyquist sampling xyz/Zeiss LSM 710 Genetically expressed GFP-tagged nuclear protein in brain slice.

**Below:** 3D rendering of nuclei stained for LamDm0 in *Drosophila* tissue, mounted in CFM3 + antifade, imaged via confocal microscopy. Notice the staining intensity is consistent throughout the entire 55  $\mu\text{m}$  stack with no increase in spherical aberration.



## ■ Citifluor CFM-1 Mountant Solution

### Usage

This glycerol-phosphate buffered saline based solution has been specially formulated so as to have a refractive index of ~1.52 (at room temperature). This refractive index will be a close match to that of the glass of the objective lens and cover-slip. The CFM-1 solution will be particularly useful for high magnification work where immersion oils are used to minimise distortion of the image due to refraction of the viewing light. The solutions should also be particularly valuable where three-dimensional imaging of specimens is carried out using confocal microscopy.

A few drops of the solution should be applied to the specimen followed by a cover slip. An immersion oil (which may be CFM solution if its viscosity is appropriate) is applied in the usual way.

### Properties and Storage

The solutions are of medium viscosity, are water-white in appearance and have a pH of ~7.5. They may be



stored at room temperature. The cap of the bottles should always be replaced after use to prevent evaporation of water. Samples stored under these conditions for 6 months have been found to exhibit little apparent deterioration.

	Cat No.	Description	Qty.
RT	<b>17980-10</b>	Citifluor CFM-1	10 ml
RT	<b>17980-25</b>	Citifluor CFM-1	25 ml

## ■ Citifluor CFM-1 Plus Antifadent Mountant Solution

### Usage

This glycerol-phosphate buffered saline based solutions has been specially formulated so as to have a refractive index of ~1.52 (at room temperature) and contains an antifadent to retard the bleaching of fluorochromes. The refractive index of the solution is designed to match the glass of the objective lens and coverslip. The CFM 1 Solution Plus Antifadent will be particularly useful for high magnification work where immersion oils are used to minimise distortion of the image due to refraction of the viewing light and where bleaching of the fluorochrome occurs. The solution should also be very valuable for three-dimensional imaging of specimens using confocal fluorescence microscopy where integrity of the image has to be maintained. To obtain images at some depth within the tissue it may be necessary to reduce the refractive index of the supplied solution. This may be done by adding sufficient glycerol to render clarity.

A few drops of the solution should be applied to the specimen followed by a cover slip.



### Properties and Storage

The solution is of medium viscosity, has a water-white in appearance and a pH of ~9. The solution should be stored at room temperature. The cap of the bottles should always be replaced after use to prevent evaporation of water. Samples stored under these conditions for 6 months have been found to exhibit little apparent deterioration.

	Cat No.	Description	Qty.
RT	<b>17981-10</b>	Citifluor CFM-1 + Antifadent	10 ml
RT	<b>17981-25</b>	Citifluor CFM-1 + Antifadent	25 ml

# Non-Hardening Mountant Solutions

Glycerol-Based, High Refractive Index Solutions

## ■ Citifluor CFM-2 Mountant Solution

### Usage

This glycerol / tris buffered based solution has been specially formulated so as to have a refractive index of ~1.52 (at room temperature) which should be a close match to the refractive index of the glass of the objective lens and cover-slip. The solution has a pH of ~8.5 which is appropriate for fluorescein conjugates. The CFM solutions will be particularly useful for high magnification work where immersion oils are used to minimise distortion of the image due to refraction of the viewing light. The solutions should also be particularly valuable where three-dimensional imaging of specimens is carried out using confocal microscopy.

A few drops of the solution should be applied to the specimen followed by a cover slip.

### Properties and Storage

The solution is of medium viscosity, having a water-white in appearance and a pH of ~8.5. It may be stored at room temperature. The cap of the bottles should always be replaced after use to prevent evaporation of water. The solution should be stored at room temperature and out of strong sunlight. Under these conditions solutions have been found to exhibit little apparent deterioration over a 6 month period.

	Cat No.	Description	Qty.
RT	17982-10	Citifluor CFM-2	10 ml
RT	17982-25	Citifluor CFM-2	25 ml



## ■ Citifluor CFMR2 Mountant Solution

A high refractive index mountant specially designed for samples labeled with GFP

The CitiFluor™ CFM series have proved very useful for imaging materials using CLSM. Samples labeled with GFP pose a special problem since the retention of a good image during imaging and upon storage is dependent upon the mountant solution containing oxygen. The classical antifadents consume oxygen when the fluorochromes are irradiated and in the case of GFP this accelerates the loss of the image.

CFMR2 contains a unique antifadent that does not de-oxygenate solution but nevertheless, affords protection to the GFP.

As with the other CitiFluor™ CFM solutions the mountant has a similar refractive index to the biological tissue and hence enables a good depth of viewing within the sample.

	Cat No.	Description	Qty.
RT	17979-10	Citifluor CFMR2	10 ml



## ■ Citifluor CFM-3 Mountant Solution

### Usage

This glycerol based solution has been specially formulated so as to have a refractive index of ~1.52 (at room temperature) and contains an antifadent to retard the bleaching of fluorochromes. The refractive index of the solution should match the refractive index of the glass of the objective lens and glass of the cover-slip. The CFM-3 solutions will be particularly useful for high magnification work where immersion oils are used to minimize distortion of the image due to refraction of the viewing light and bleaching of the fluorochrome occurs. The solutions should also be very valuable for three-dimensional imaging of specimens using confocal fluorescence microscopy, where integrity of the image has to be maintained since the CFM-3 not only minimizes the effects of refraction, but it also allows visualizing fluorochromes present at depth within the sample. If your sample doesn't appear clear when viewed with white light, cautiously add small amounts of glycerol until clarity is obtained.

Since the CFM-3 solution has a relatively low pH solution, wash the sample with a buffer of appropriate pH followed by a couple of washes with the CFM-3 solution. Apply a cover slip.

### Properties and Storage

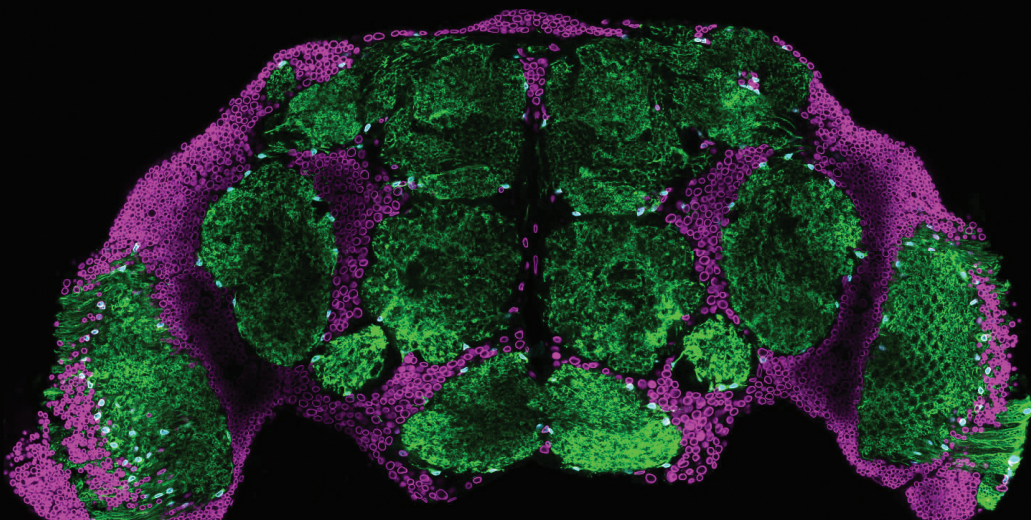
The solutions are of medium viscosity, are water-white in appearance and have a pH of ~6.5. The CFM-3 solution should NOT BE STORED IN A REFRIGERATOR but at room temperature and out of strong sunlight. The cap of the bottles should always be replaced after use to prevent evaporation of water. Solutions stored under these conditions have been found to exhibit little apparent deterioration over a 6 month period, although occasionally small crystals may form. These may be removed by either centrifugation or filtration.



**Below:** Drosophila brain mounted in CFM3: fluorochromes, Dylight 488 (pseudo-colored green), Dylight 549 (pseudo-colored magenta), and Dylight 649 (pseudo-colored cyan).

Courtesy Dr. Sean Speese  
OHsu, USA.

Cat No.	Description	Qty.
RT 17979-20	Citifluor CFM-3	10 ml
RT 17979-30	Citifluor CFM-3	25 ml



# Non-Hardening Mountant Solutions

## Glycerol-Free Solutions

### ■ Citifluor AF3 Mountant Solution

#### Usage

AF3 is a mountant solution composed of phosphate buffered saline and an antifadent. It was specifically designed to stop the photobleaching of the fluorescein moiety of FITC labelled biological specimens. Its application is not however limited to FITC labelled materials and has been used with advantage with many other fluorochromes including rhodamines, Alexa dyes and DAPI. It is useful for examining tissue live cells although it should be noted that cell lysis will take place over a period of time. If this is a problem, use our AFR3 solution.

The solution has a pH of ~10. The solution should be pipetted onto the specimen and then a cover slip applied. Specimens mounted in AF3 solutions have been kept in this way for many months without suffering damage.

#### Properties and Storage

The solutions have a water-white in appearance. They may be stored at room temperature and ideally between 50 and 150 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 as a matter of good practice. Samples stored under these conditions for 6 months have shown no apparent deterioration. If the AF3 solution is being used in an assay, a control experiment should always be carried out.



	Cat No.	Description	Qty.
<input type="checkbox"/> RT	17972-25	Citifluor AF3	25 ml
<input type="checkbox"/> RT	17972-100	Citifluor AF3	100 ml

### ■ Citifluor AFR3 Mountant Solution

#### A new antifadent solution for use with live cells

The imaging of live cells in conjunction with an antifadent poses problems. The classical antifadents, amines and phenols can interact with the cell surface leading to lysis. The new solution contains an antifadent at a much lower concentration than that used is in the currently available solutions and is designed to show minimal phototoxicity. Unlike other antifadents it does not de-oxygenate the solution. The antifadent is supplied in a phosphate buffered saline solution. Texas Red, Alexa and Cyanine dyes have been stabilized with the new antifadent. This solution has a refractive index of 1.34 and hence is ideally suited for use with Total Internal Reflectance Microscopy (TIRFM).



	Cat No.	Description	Qty.
<input type="checkbox"/> RT	17973-10	Citifluor AFR3	10 ml

## ■ Citifluor CFPVOH Plus AF100 Mountant Solution

### Usage

CFPVOH plus antifadent is an aqueous solution of poly (vinyl alcohol) containing an antifadent and is intended for use where it is desired to have a permanent mountant for a specimen which has been fluorescently labeled. The antifadent helps retard bleaching of fluorescent label with the extent of retardation being very dependent upon the type of label and the molecular structure of the specimen.

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.

If bleaching of the label remains unacceptably high, try a mixture of CFPVOH plus antifadent with AF100 mixed in the ratio of 10:1 (v/v). This solution will have a short shelf life (a few days).

### Properties and Storage

The solutions are of medium viscosity, are water-white in appearance and have a pH of ~8.5. They contain a small amount of sodium azide to prevent fungal growth. They may be stored at room temperature. The cap of the bottles should always be replaced after use to prevent evaporation of water, which leads to film formation and ultimately to solidification of the product. Samples stored under these conditions for 2 months have been found to exhibit little apparent deterioration, e.g. no gel formation.

Cat No.	Description	Qty.
RT 17978-35	Citifluor CFPVOH + AF100*	25 ml + 5 ml

\* Only available when ordering with CFPVOH

## ■ Citifluor AF87 Mountant Solution

### High Refractive Index

### Usage

AF87 is a non-fluorescent immersion oil of medium viscosity and has a refractive index of 1.516 and may be used as an immersion oil or as a mountant medium.

If used as an immersion oil it is recommended that the microscope objective is wiped clean after use. A useful solvent mixture for removing the immersion oil is composed of ether / ethanol (7:3 v/v) or alternatively, xylene may be used.

To use AF87 as a mountant medium it is necessary to dehydrate the sample prior to application by washing it with successive amounts of absolute ethanol prior to drying. Once the sample is dry the AF87 may be applied. The AF87 contains an antifadent to reduce the amount of fluorochrome fading. AF87 has been found useful for examining specimens generated using the technique of FISH, labeled with the fluorochromes DAPI and Cy dyes.

### Properties and Storage

The immersion oil may be stored at 5° to 100°C and should be kept out of strong sunlight. It should NOT BE STORED IN A REFRIGERATOR. Occasionally small crystals are formed in the oil, but careful use of a pipette can obviate the crystals interfering with the function of the oil.

Cat No.	Description	Qty.
RT 17976-10	Citifluor AF87	10 ml
RT 17976-25	Citifluor AF87	25 ml



# Hardening Mountant Solutions

## Glycerol-Based Solutions

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 and CFPVOH.

### ■ Citifluor AF200 Mountant Solution

#### Usage

AF200 is a solution of and antifadent in glycerol and has been specially prepared for use with MWL 4-88 solutions where DAPI is the staining material. The solutions may be used with other fluorochromes such as fluorescein, Alexa dyes and Hoechst dyes. The presence of the antifadent helps to reduce photobleaching of the fluorophore. Solutions should be made up by mixing 1 part by volume of AF200 with 3 parts by volume of MWL 4-88. These mixtures should be used within 10 hours as the efficacy of the antifadent reduces with time. A few drops of the solution should be applied to the specimen followed by a cover slip. When used with MWL 4-88, evaporation of the water leaves a clear film which holds the cover slip in place, thereby aiding the safe storage of the specimen.

#### Properties and Storage

The solution is of medium viscosity and has a water-white appearance. The AF200 / MWL 4-88 solutions do not keep and the efficacy of the antifadent is markedly reduced over a 10 hour period. With this in mind, it is better to make up the mixture prior to use and not to rely on keeping solutions.



	Cat No.	Description	Qty.
RT	17977-10	Citifluor AF200	10 ml

### ■ Citifluor AF300 Mountant Solution

#### Usage

AF300 is a solution of and antifadent in glycerol and has been specially prepared for use with MWL 4-88 solutions where fluorescein is the staining material. The solutions may be used with other fluorochromes such as rhodamines, etc. The presence of the antifadent helps to reduce photobleaching of the fluorophore and to enhance the rate of solidification. Solutions should be made up by mixing 1 part by volume of AF300 with 3 parts by volume of MWL 4-88. A few drops of the solution should be applied to the specimen followed by a cover slip.

#### Properties and Storage

The solution is of medium viscosity and has a water-white appearance. The AF200 / MWL 4-88 solutions do not keep and the efficacy of the antifadent is markedly reduced over a 10 hour period. With this in mind, it is better to make up the mixture prior to use and not to rely on keeping solutions.

	Cat No.	Description	Qty.
RT	17977-25	Citifluor AF300	10 ml



## ■ Citifluor Tris-MWL 4-88 Mountant Solution

### Usage

Tris-MWL 4-88 solution is a solution of poly (vinyl alcohol), Mowiol® 4-88, in a water/glycerol/tris buffer mix and has a pH of 8.5. Following loss of water it forms good clear films and is therefore used as a permanent mountant. 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.

### Properties and Storage

The solutions are of medium viscosity, are water-white in appearance and have a pH of ~8.5. They may be stored at room temperature. The cap of the bottles should always be replaced after use to prevent evaporation of water, which leads to film formation and ultimately to solidification of the product. Samples stored under these conditions for 6 months have been found to exhibit little apparent deterioration, e.g. no gel formation.

If you want a solution of higher viscosity, we can make these to order.

If bleaching of the fluorescent marker is a problem, you may add AF100 as you would to CFPVOH (i.e. 9 parts MWL solution plus 1 part AF100). These mixtures have a limited shelf-life.



	Cat No.	Description	Qty.
RT	<b>17977-150</b>	Citifluor MWL4-88	25 ml

## ■ Citifluor CFPVOH and AF100 Mountant Solutions

### Usage

CFPVOH plus antifadent is an aqueous solution of poly (vinyl alcohol) containing an antifadent and is intended for use where it is desired to have a permanent mountant for a specimen which has been fluorescently labeled. The antifadent helps retard bleaching of fluorescent label with the extent of retardation being very dependent upon the type of label and the molecular structure of the specimen.

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.

If bleaching of the label remains unacceptably high, try a mixture of CFPVOH plus antifadent with AF100 mixed in the ratio of 10:1 (v/v). This solution will have a short shelf life (a few days).

### Properties and Storage

The solutions are of medium viscosity, are water-white in appearance and have a pH of ~8.5. They contain a small amount of sodium azide to prevent fungal growth. They may be stored at room temperature. The cap of the bottles should always be replaced after use to prevent evaporation of water, which leads to film formation and ultimately to solidification of the product. Samples stored under these conditions for 2 months have been found to exhibit little apparent deterioration, e.g. no gel formation.

	Cat No.	Description	Qty.
RT	<b>17978-35</b>	Citifluor CFPVOH + AF100*	25 ml + 5 ml

\* Only available when ordering with CFPVOH

# Hardening Mountant Solutions

## Glycerol-Based, Film-Forming Polymer Solutions

### ■ Citifluor PVP-1 and PVP-1 Plus Antifadent Mountant Solutions



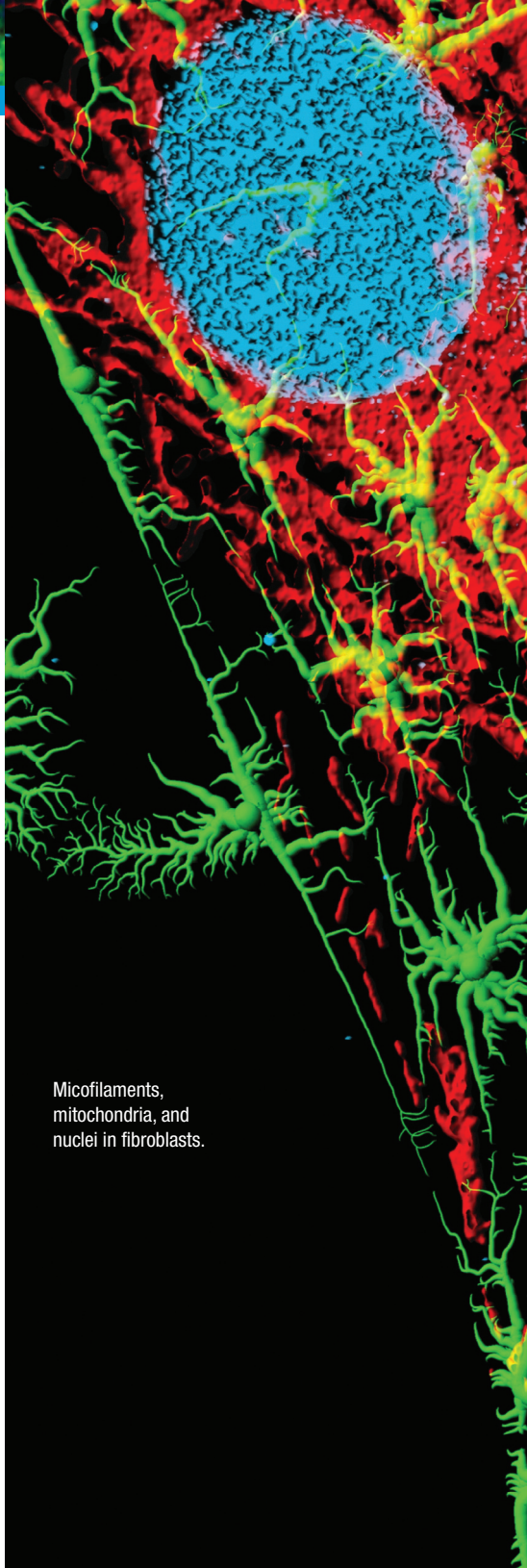
### Usage

Aqueous poly (vinyl pyrrolidone) plus antifadent is very useful as a permanent mountant which possesses antifadent properties. 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 having a high refractive index (approaching 1.5 – the value depends on the extent to which water is removed). The dried film the cover slip in place, thereby aiding the safe storage of the specimen.

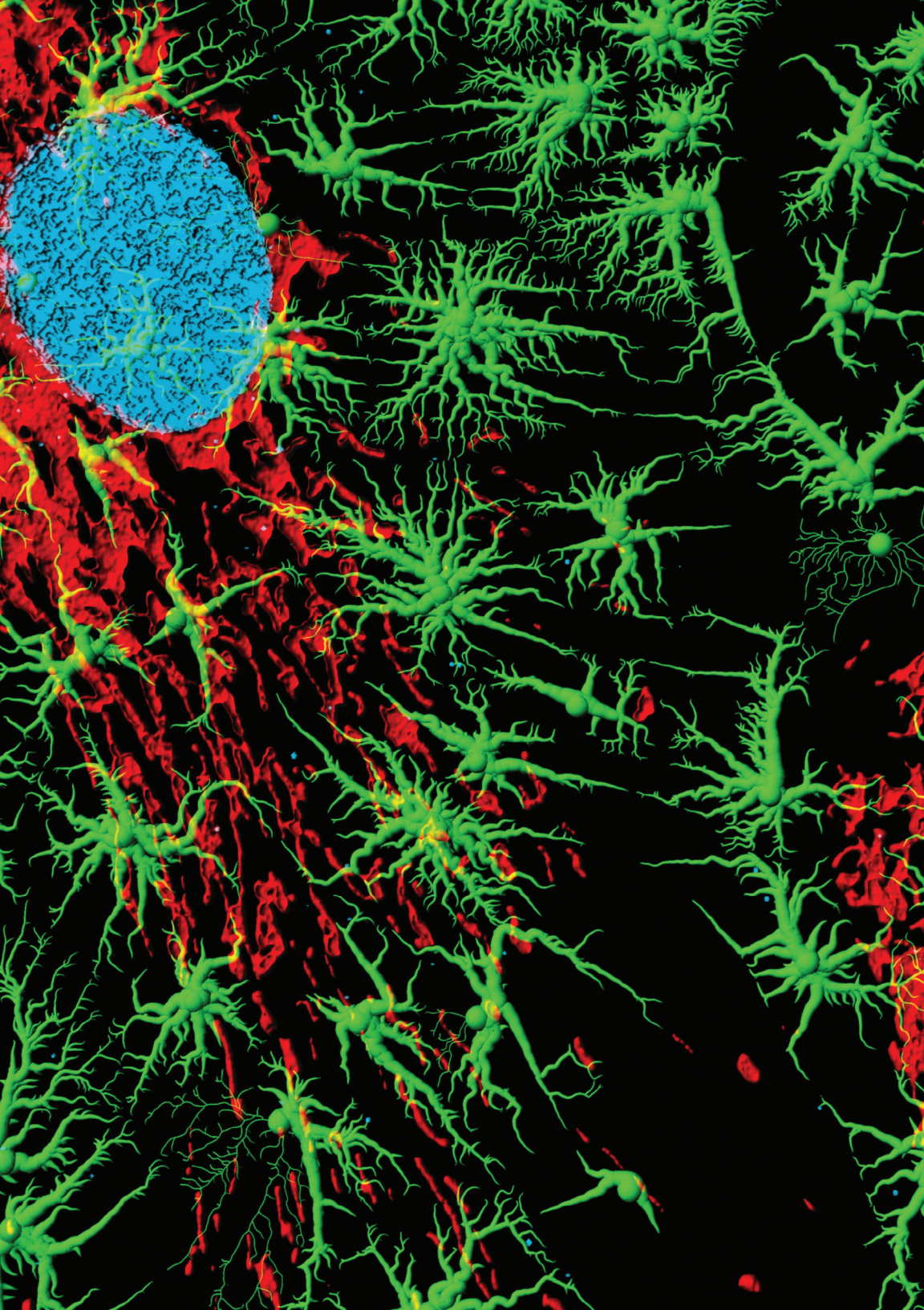
### Properties and Storage

The solutions are of low viscosity and are slightly yellow in appearance. They have a pH of ~9.5. The solutions may be stored at room temperature. The cap of the bottles should always be replaced after use to prevent evaporation of water, which leads to film formation and ultimately to solidification of the product. Samples stored under these conditions for 6 months have been found to exhibit little apparent deterioration, e.g. no gel formation.

	Cat No.	Description	Qty.
RT	17977-50	Citifluor PVP-1	25 ml
RT	17977-100	Citifluor PVP-1 + Antifadent	25 ml



Micofilaments,  
mitochondria, and  
nuclei in fibroblasts.



Paper Ref.No.	Year	Technique	Area of Application	Fluorochrome	Fluorochrome	Fluorochrome	Comments/Notes
<b>1</b>	2009	CLSM	Neuroscience	DAPI		FITC	Chagas disease
<b>2</b>	2007	CLSM	Neuroscience	FITC			
<b>3</b>	2005	FISH	Oceanography		TRITC	FITC	Coral
<b>4</b>	2005	FISH	Genetics	DAPI		FITC, Texas Red	TR Avidin
<b>5</b>	2007	FISH	Bacteria	DAPI			rRNA
<b>6</b>	2012	FISH	Physiology	DAPI			
<b>7</b>	2010	FISH	Medicine	DAPI	GFP		
<b>8</b>	2010		Surgery, Neuroscience	DAPI		Alexa 488, Alexa 594	
<b>9</b>	2012	FISH	Genetics	DAPI		Alexa 488	
<b>10</b>	2012		Bacteriology	DAPI			4:1 CF plus Vectashield
<b>11</b>	2009	FISH	Reproduction	Hoechst 33258		FITC, rhodamine-phalloidin	
<b>12</b>	2008		Ophthalmology	rhodamine	Alexa fluor 633		
<b>13</b>	2005		Medical	rhodamine			
<b>14</b>	2007		Medical	rhodamine			Reproduction, calcium ions
<b>15</b>	2010		Medical	TRITC			Lung research
<b>16</b>	2011		Cell biology	TRITC			
<b>17</b>	2010	FISH, PCR	Bacteriology	rhodamine	fluorescein		
<b>18</b>	2010		Medicine	Hoechst			Reproduction, cAMP
<b>19</b>	2010		Medicine	Hoechst 33258	Alamar Blue		Alzheimers
<b>20</b>	2011		Reproduction	Hoechst			Chromatin
<b>21</b>	2011			Hoechst 33258			AF1
<b>22</b>	2012		Medicine	Hoechst 33258			Wound healing
<b>23</b>	2012	FISH	Bacteriology, Oceanography	Hoechst?			AF1
<b>24</b>	2011		Reproduction	Hoechst			Effect of smoking
<b>25</b>	2010		Ophthalmology	DAPI		Alexa Fluor 594, Alexa Fluor 488	Histology
<b>26</b>	2009		Microbiology		GFP	Alexa 594	
<b>27</b>	2011		Pharmacy			Alexa 488	
<b>28</b>	2011	CARD-FISH	Bacteriology			Alexa 488	Used with Vectashield
<b>29</b>	2011	CLSM	Virology	FITC		Alexa 546	
<b>30</b>	2010	CLSM	Histology	Cy 3	DyLight 488	FITC	Zebra Fish
<b>31</b>	2011		Medicine	C 3, Cy 5		Alexa 488	
<b>32</b>	2010		Neuroscience	Cy 3			
<b>33</b>	2009		Microbiology	Cy 3			Volcanic lakes
<b>34</b>	2009	FISH	Genetics	Cy3, Cy5			rRNA labelling
<b>35</b>	2010	FISH	Microbiology	Cy3, DAPI			Methane producing bacteria, sulfate reducing bacteria
<b>36</b>	2012	CLSM-FISH	Microbiology		Cy3 and Cy5		
<b>37</b>	2010	FISH	Microbiology	DAPI FITC	Cy3		Excellent general text
<b>38</b>	2009	FISH	Physiology			Texas Red	Labelled antibody
<b>39</b>	2008		Surgery			Texas Red	
<b>39</b>	2008	CLSM	Surgery, Neuroscience		GFP		Spinal cord injury, photo-stimulation of neurons
<b>40</b>	2008		Brain Research			Texas Red	
<b>41</b>	2008		Neuroscience		GFP	Texas Red	
<b>42</b>	2006		Immunology	FITC	Cy3	Texas Red	
<b>43</b>	2007		Neuroscience	Phycocerythrin			
<b>44</b>	2008		Neuroscience	R-phycoerythrin			
<b>45</b>	2004		Medicine	DAPI	Streptavidin-phycoerythrin conjugate		
<b>46</b>	2009		Microbiology	Phycocerythrin			Autofluorescence
<b>47</b>	2008		Neuroscience	FITC	Phycocerythrin conjugate		
<b>48</b>	2007		Medicine	FITC	Phycocerythrin conjugate		
<b>49</b>	2009		Neurology	Hoechst 33342	GFP		
<b>50</b>	2008	CLSM	Virology		GFP	YFP, CFP	Yellow and cyan FP
<b>51</b>	2010		Physiology	Hoechst 33342	GFP		
<b>52</b>	2008	CLSM	Bacteriology	Cy3	GFP		
<b>53</b>	2008		Neurology		GFP		
<b>54</b>	2010	CLSM	Microbiology		GFP		
<b>55</b>	1985		Bacteriology		Acridine orange		
<b>56</b>	1990		Microbiology	Phycocerythrin autofluorescence			
<b>57</b>	2007		Bacteriology	DAPI	Phycocerythrin		Autofluorescence
<b>58</b>	2008	FISH	Microbiology				75% AF1, 10% VectashieldH- 1000

Paper Ref.No.	Year	Technique	Area of Application	Fluorochrome	Fluorochrome	Fluorochrome	Comments/Notes
<b>Citifluor AF1 Applications (continued)</b>							
59	2008	FISH		DAPI			PNA
60	2011	FISH	Medicine				
62	2002	FISH	Bacteriology	DAPI	Cy3		5.5 parts AF1, 1 part Vectashield 0.5 parts PBS
63	2002	CARD-FISH	Bacteriology				Good explanation of CARD FISH labelled nucleotides
64	2011	CARD-FISH	Bacteriology				4:1 CF plus Vectashield
65	2010	CARD-FISH	Bacteriology			Catalysed reporter deposition	4:1 CF plus Vectashield
66	2011	FISH	Bacteriology				5.5CF plus1 Vectashield plus 0.5 PBS

## Citifluor AF2 Applications

70	2012	CLSM	Botany			Alexa 488	
70	2012	CLSM	Botany	FITC	DAPI		
71	2011	CLSM	Food Science	FITC	rhodamine B		
72	2006		Botany	Cy3	FITC		
73	1998	CLSM FISH	Bacteriology	DAPI	FITC	Cy 3	
74	2009		Water quality	DAPI			
74	2009		Bacteriology		GFP		
75	1998	CLSM	Medicine	DAPI			Fertilization
76	2012	CARD FISH	Bacteriology	DAPI			Cell counting microscope
77	2009	FISH	Astrobiology	DAPI	Cy3		
79	2012	CARD FISH	Bacteriology	Cy 3 tyramide	GFP		
80	2012		Botany		GFP		
81	2011		Bacteriology		GFP		
82	2007		Bacteriology		GFP		
83	2012	FISH	Astrobiology	Cy3			
84	2003		Medicine	FITC			Use AF2 in solid mountant

## Citifluor AF3 Applications

86	2005	FISH		FITC		Immunogold	rRNA
87	2006						
88	2009		Bacteria			Alexa Fluor tyramide	
88	2009		Microbiology				
88	2009	CLSMFISH	Dentistry				rRNA
89	2011	CLSMFISH	Dentistry			Alexa 488	
89	2011		Dentistry				
90	2012	CARD FISH	Bacteria	DAPI			rRNA
91	2011	CLSM	Genetics	DAPI		Alexa488 594	
92	2010		Drug Release	DAPI			
93	2009	CLSMFISH	Dentistry				
94	2006		Water Quality	Carboxyfluorescein	TRITC		
95	2011		Neurology		Alexa 488, 568, 633		
95	2011		Toxicology				
96	2002	FISH	Limnology	DAPI		roscoff	Tyramide signal amplification rRNA
97	2012	FISH	Physiology				Calcium deposits in crustaceans
98							
99							

## Citifluor AF87 Applications

100	2003	CLSMFISH	Bacteriology				
101	2005		Botany				Alga
102	2003	FISH	Bacteriology		Cy3		Method for using AF87
103	2005			DAPI	Cy3		
104	2005			DAPI			
105	2009	FISH	Bacteriology	DAPI			

## Citifluor CFPVOH/AF100 Applications

106	2008	CLSM	Ophthalmology	Alexa 488			AF100
107	2010	CLSM	Medicine	FITC	DAPI	GFP	AF100 Huntingdons
108	2008	FISH	Microbiology	FITC	Cy3	Cy5	AF100
109	2005		Neuroscience	Hoechst 2495	Alexa 488		AF100
110	2009		Microbiology	Alexa 488			AF100
111	2007		Cell Biology	Alexa 594-phalloidin	FITC		20%Airvol 203 + 4% AF1
112	2007		Cell Biology	Alexa 594-phalloidin	FITC		20%Airvol 203 + 4% AF1

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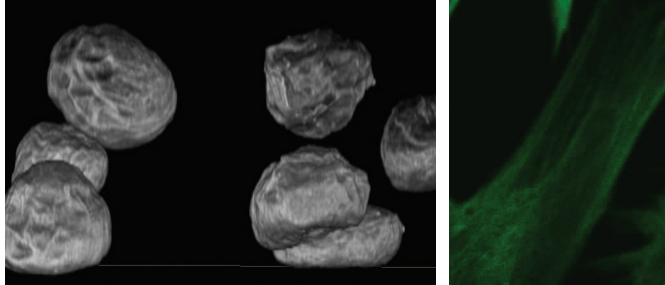
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