

TECHNICAL DATA SHEET 124

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Glutaraldehyde, EM Grade

For High Purity EM and Biological Use

Background

Glutaraldehyde is one of the most widely used fixatives for Electron Microscopy and general biological applications. Polysciences pioneered the supply of redistilled high purity glutaraldehyde and has constantly improved the product so that it is now the standard used by discriminating researchers. We offer several concentrations of the high purity EM Grade and a biological grade for routine work.

Glutaraldehyde, like any aldehyde, is subject to easy air oxidation and polymerization when in concentrated solutions of 25% or above. Both reactions result in loss of aldehyde tissue fixation and inhibit enzymes. The initial reports of Sabatini and Barnett¹ stated that high purity glutaraldehyde was essential for optimum fixation. In order to provide a reproducible, standardized glutaraldehyde, free of contaminants, we offer highly purified 8%, 25%, 50%, 70% glutaraldehyde solutions of EM Grade.

Purification

Our method of purification starts with a technical grade glutaraldehyde which is then glass distilled at low temperatures under vacuum in a multiple plate column to minimize polymerization. Subsequent steps of column purification and assay by aldehyde titration, ultraviolet spectroscopy and refractive index insure reproducibility of each batch.

Packaging

For your safety, glutaraldehyde is packaged in pre-scored ampoules. Each ampoule is packed with an ampoule cracker for an added measure of safety. The distilled EM Grade glutaraldehyde is diluted with distilled water to 8%, 25%, 50%, 70%, and adjusted to pH of approximately 5.5. The glutaraldehyde is packaged in neutral, glass ampoules and stored at 4°C. The prescored glass ampoule can be easily snapped open without any tools and added to the required buffer. Our laboratory data indicates that, after 6 months in storage at 4°C, there is no change in fixation effectiveness.

Chemical Stability

Commercial glutaraldehyde is a mixture of monomeric dialdehyde and varying amounts of impurities attributable to polymerization. Pure glutaraldehyde has an absorbance peak at 280nm, while the polymeric materials absorb at 235nm. A study on the influence of pH and temperature on polymerization of glutaraldehyde², which parallels our own program to develop the highest grade EM glutaraldehyde, has conclusively

shown that the best stability for monomeric glutaraldehyde is found at approximately pH 5.5 at 4°C. (see *Buffers)

The 8% glutaraldehyde solution breaks down autolytically into glutaric acid, causing the pH to fall below optimum level, and results in a poor quality of fixation. At high concentrations, the solutions are also subject to polymerization to viscous polymers which alter the rate of infiltration.

In an unbiased study comparing glutaraldehyde from six prominent suppliers the Polysciences' product was the only one which showed practically no contamination by undesirable polymeric products.³ A recent independent quality control study concluded that no polymers and no impurities at 235nm were present in unopened, refrigerated ampoules, even after four years of storage.⁴

Buffers

The optimum buffer will vary with the tissue and the type of tissue study to be made. Powell⁵ and his associates, using several widely used buffer systems, emphasize the importance of reporting the molality, pH, and osmolality of the fixing solution. Our glutaraldehyde, EM Grade, is free of extraneous compounds and no alteration of your fixing procedure should be necessary when you buffer this product to your required pH.

To make pH 7.0 glutaraldehyde, for example, it is merely necessary to dilute our glutaraldehyde with a buffer having

this pH. Our purified glutaraldehyde stored at pH 5.5 has negligible acidity and polymer and yields reproducible results. Glutaraldehyde polymerizes rapidly at high pHs. At pH 8.5 or over, for instance, polymerization occurs so rapidly, even at 4°C, that it is advisable not to mix the buffer and fixative until immediately prior to use. Approximately 50% polymerization of purified glutaraldehyde occurs at 4°C in seven weeks at pH 6.5, in three weeks at pH 7.5, and in six days at pH 8.5. The desired pH can be obtained by the addition of a few drops of dilute solution HCl. It should be noted that the rate of glutaraldehyde polymerization differs in various buffers at the same pH. Less polymerization occurs when glutaraldehyde is stored in cacodylate buffer compared with that in phosphate buffer at the same pH (7.4).¹⁹

The dimeric α , β -unsaturated aldehyde aldol condensation product absorbing at 235nm, may be desirable especially for morphologic preservation. As opposed to the other polymeric products which are poor fixatives and inhibit enzymes,⁶ it is readily formed in pure glutaraldehyde solutions under fixation conditions. The formation of this compound in pure glutaraldehyde solutions is apparently catalyzed by both plant and animal tissues during fixation.⁷

Fixation

Our vials contain 10 ml of 8% solution and once the ampoule is opened, it should be used within a few days. Fresh, reproducible solutions can thus be made up frequently, without undue waste, as each vial is low in cost. Double fixation with glutaraldehyde followed by osmium tetroxide is recommended for added contrast and greater stabilization in microscopic examination.^{8,9} Polysciences also supplies OsO₄ in a safe, easily handled snap-open vial, as well as all embedding media.

Proteins are very effectively cross-linked by glutaraldehyde.¹⁰ Peters and Richards¹¹ suggested that Schiff base formation by reaction of the aldehyde with an amino group of enzyme is not sufficient to explain all the observed facts regarding reaction with crystalline enzymes. They assume the formation of an α - β unsaturated aldehyde. This intermediate can cross-link with protein crystals.

Glutaraldehyde is an effective chemical sterilizer and has been used extensively in hospitals for this purpose. Its advantages over formaldehyde include its mild odor and the fact that it is less irritating to mucous membranes and the skin.

Preservation of Enzyme Activity and Cellular Ultrastructure

Glutaraldehyde is a dialdehyde and reacts rapidly with proteins in a manner analogous to formaldehyde with the first steps involving the free amino groups.¹² Many investigators consider glutaraldehyde the best of the aldehydes tested.^{3,5,13} Glutaraldehyde was shown to be more active than acetaldehyde or formaldehyde in fixing erythrocyte membrane.¹⁴

Immunochemistry

Glutaraldehyde has been used as a coupling agent to prepare peroxidase coupled antibody, which is used for localization of antigen at the Electron Microscopic level.^{15, 16} The two-step procedure is generally preferred over the one-step method.¹⁷ Ferritin, an electron dense iron containing protein, can be coupled to antibody with glutaraldehyde¹⁶ for use in antigen localization under EM.

Glutaraldehyde fixation does not decrease RBC agglutination by lectins^{18,19} and allows them to retain their sensitivity to hemagglutination and hemagglutination inhibition tests for arbovirus antigens and antibodies.^{19,21} Glutaraldehyde is a very effective fixative for ribosomes and polyribosomes *in vitro*.^{19,22}

EM Grade ferritin is also available from Polysciences. Glutaraldehyde is available as 8%, 25%, 50%, and 70% EM Grade and as 25% and 50% biological grade.

Storage and Handling Precautions Danger! Causes Burns!

Harmful if absorbed through skin. Do not get in eyes or on skin or on clothing. Avoid breathing vapors. Wash thoroughly after handling. Use only with adequate ventilation. Open vial only under a hood. Wear protective gloves and safety goggles. Store at 4° to 8°C (39°-46°F). Keep container closed. Our EM Grade Glutaraldehyde is stable for one year at 4°C in sealed ampoules.

First Aid

In case of contact immediately flush eye or skin with plenty of water for at least 15 minutes. Remove contaminated clothing and shoes. Call a physician. Wash clothing before reuse. See Material Safety Data Sheet for further information.

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation.

Ordering Information:**Glutaraldehyde, EM Grade**

Cat.#	Description	Size
00216	Snap-open vials, 8% aqueous	30 x 10 ml amp.
00216A	8% aqueous	10 x 10 ml amp.
07710	8% aqueous	100 ml 5 x 100 ml
01909	25% aqueous	100 ml 10 x 10 ml amp.
18428	50% aqueous	100 ml 10 x 10 ml amp.
01201	70% aqueous	5 x 10 ml amp. 10 x 2 ml amp.

Glutaraldehyde, Biological Grade

Cat.#	Description	Size
00376	25% aqueous	500 ml 4 x 1000 ml
00377	50% aqueous	500 ml 4 x 1000 ml

To Order:

In The U.S. Call: 1-800-523-2575 • 215-343-6484

In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767

In Germany FAX: (49) 6221-764620

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