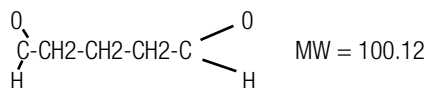


Glutaraldehyde Fixative

ALDEHYDE

- A. Glutaraldehyde (Sabatini, Bensch & Barrnett, 1963)



ADVANTAGES

1. Enzymatic activity partially preserved which permits cytochemical localization following fixation.
2. Penetration rate slightly faster than osmium tetroxide.
3. Exerts some osmotic effect. Effect is dependent on concentration and specimen type. No significant changes observed by changing concentration of glutaraldehyde between 2% and 5%.

DISADVANTAGES

1. Does not destroy osmotic properties of cells. Osmolarity of fixative must be carefully controlled.
2. Can cause shrinkage of cells at higher concentrations.
3. Tends to polymerize at alkaline pH (above 7.5).
4. Has no staining action.
5. Reacts only slightly with lipids. May cause phospholipids to pass into solution producing myelinic figures upon reaction with osmium tetroxide. Myelinic figures can be prevented by adding 1-3mM CaCl₂ to glutaraldehyde fixative, but care must be taken since Ca²⁺ ions may precipitate proteins.

COMMERCIAL AVAILABILITY

1. Bulk quantities: solutions (usually 25 or 50%). Contain impurities and polymers of glutaraldehyde. Must be purified.
 - a. Activated charcoal- 10% (w/v). Shake 1 hr at 4° C. Filter. Repeat 2 or 3 times.
 - b. Distillation. Collect distillate between 100° and 101° C in 50 ml aliquots. Discard those with pH below 3.4.
 - c. Vacuum distillation. Distill at 15 mm mercury pressure. Collect distillate at 80-85° C and immediately dilute to 25% with boiling water.
2. Solutions of vacuum distilled glutaraldehyde (usually 50 or 70%) sealed under nitrogen.

STORAGE

Stable for many months if stored at -20° C.
May be stored for shorter time at 4° C.

CHARACTERISTICS OF PURE GLUTARALDEHYDE

1. Clear. A yellow color indicates polymerization. Color should be no more than pale yellow.
2. pH above 3.5. Should be discarded if pH is below 3.5.
3. Spectrophotometric absorption at 280 nm. Other absorption peaks indicate impurities (usually absorb at 235 nm).

PREPARATION OF FIXING SOLUTIONS

Should be prepared and stored in clean, glass-stoppered bottles.
Stable for some weeks at 4° C in a refrigerator in the dark.
Freshly prepared final solutions should be used whenever possible.

STOCK SOLUTIONS

Glutaraldehyde: Dilute glutaraldehyde to concentration twice that of desired final conc. (usually 2-6%) with ddH₂O.

Buffer: Prepare buffer solution with concentration twice that of desired final concentration (usually 0.05-0.2M). Adjust pH of buffer to desired pH. Adjust osmolarity of buffer to desired osmolarity with sucrose, glucose or NaCl.

WORKING FIXATIVE

Mix 1 vol. of glutaraldehyde with 1 vol. buffer solution.

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