PROVIDING SOLUTIONS:

Fixatives for Electron Microscopy

KIRSCH NOTES
PROTOCOLS & TIPS FOR MICROSCOPY

Chemical fixation of biological samples for light and electron microscopies is required to halt biochemical reactions, prevent autolysis, stabilize macromolecules, preserve delicate morphologies, and reduce artefacts which may be induced during other treatments such as dehydration. Besides these general objectives for all fixatives there are also some selected to perform specific functions, such as staining individual organelles or structures. The following is a list of the more popular fixatives, their functions, and other benefits.

Primary Fixation

Paraformaldehyde, when mixed for microscopy, forms a monoaldehyde (formalin) which penetrates rapidly and stabilizes proteins-CH_2- forming methylene bridges between amine groups. Often the fixative of choice (4% in buffer) for immunocytochemistry as it leaves antigens functional. It is often combined with glutaraldehyde as a primary fixative, see Karnovsky's below.

EM Grade Glutaraldehyde is a 5-carbon dialdehyde, which cross links protein's alpha amine sites and is the universal primary fixative (2-4% in buffer) used for morphological studies. Older glutaraldehyde, that has not been stored under dry nitrogen, may polymerize greatly reducing its cross-linking effectiveness. When used in very low concentrations (0.1%) during fixation for immunocytochemistry a blocking agent must be used to block the free aldehyde groups.

Karnovsky's is an aldehyde solution combining the lighter fixation and faster penetration rate of paraformaldehyde (1-2%) and the more complete cross-linking ability of glutaraldehyde (2-4%) for the best complete primary fixation.

Glyoxal is a 2 carbon bi-aldehyde, similar to the glutaraldehyde molecule but smaller. This fixative is much less hazardous than other aldehydes due to its low vapor pressure. It is an excellent alternative to formalin for OLM and TEM applications, especially immunocytochemistry.

Secondary Fixation

Osmium Tetroxide (OsO_4) (1-4% aqueous or buffered) is a volatile toxic heavy metal oxidizer which reacts primarily with the double bonds of unsaturated lipids such as phospholipids. The osmium, being reduced, is now metallic Os, thus adding an amplitude contrast mechanism to the tissue for TEM and added conductivity and secondary electron signal for SEM. The vapors are also used as a staining mechanism for sectioned unsaturated polymers such as butadiene.

Potassium Permanganate ($KMnO_4$) is an aqueous solution (2-4%) often used for plants, yeasts, and bacterial/viral samples, any samples with a tough outer coat. The presence of Mn also adds contrast to the tissue.

Specialty Fixatives and Additives

Uranyl Acetate is sometimes employed as a tertiary fixative after osmium treatment (2-3% aqueous or in 50% ETOH or MEOH). It fixes and stains nucleic acids and membranes but is used primarily as a positive post stain along with lead citrate. Another major use of UA is as a negative stain. It is mildly radioactive but the chemical toxicity is its most dangerous property. It is light sensitive so storage of its solution in a dark bottle and away from light is essential.

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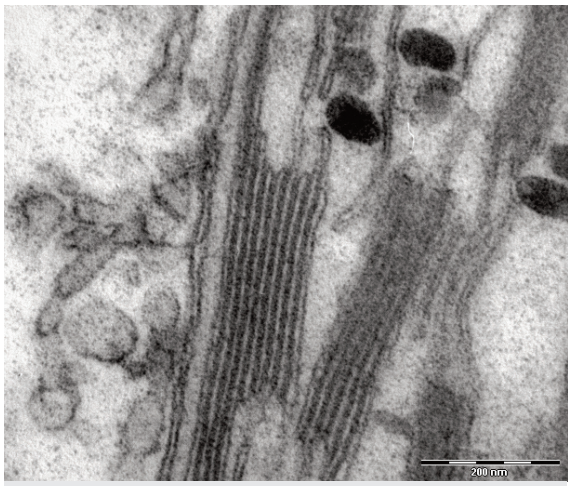
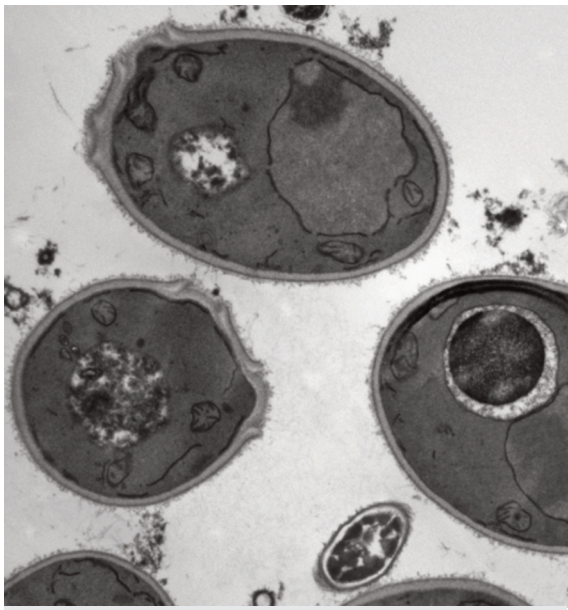


Transmission electron microscope image, showing part of a chloroplast (Anemone sp., leaf cell). The lines are grana, lamellas of thylakoids. Fixative: GA + Osmium + KFeCN (glutaraldehyde + osmium tetroxide + kaliumhexacyanoferrat). Contrast: U + Pb (uranyl acetate + lead citrate).

Image Credit: https://commons.wikimedia.org/wiki/File:Chloroplast_in_leaf_of_Anemone_sp_TEM_85000x.png#filelinks



Yeast cells were fixed with Glutaraldehyde in Cacodylate buffer, washed in distilled water and postfixed with 1% $KMnO_4$ in distilled water.

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Specialty Fixatives (continued)

Acrolein is used similarly to aldehydes when very rapid penetration is required. Also used on plants, seeds and other difficult to penetrate samples. Not in common use due to its reactivity and its propensity to form polymers.

Tannic Acid, when added to glutaraldehyde, enhances fixation of membranes and cytoskeletal fibers. It is also a mordant for heavy metal stains.

Potassium Ferrocyanide is added to Os to enhance fixation and staining of glycogen, glycoproteins and ultrastructural trabeculae between RER cisternae.

Histological Additives

Methanol/Ethanol are often added to fixatives for clinical applications to increase penetration rate and also serve as a fixative.

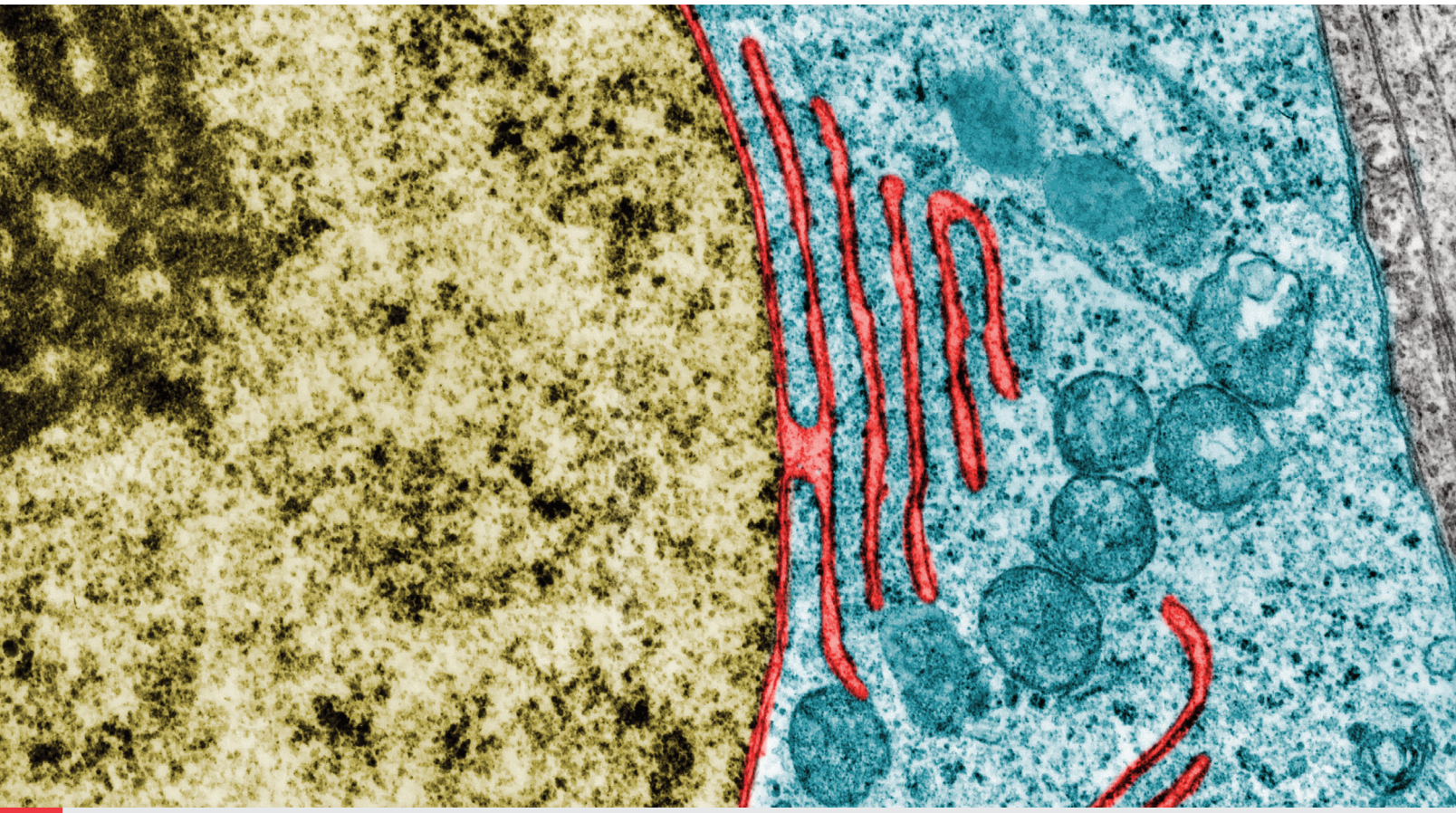
Picric Acid is used to balance the basophilic effect on the cytoplasm, caused by formalin in Bouin's fixative, resulting in excellent nuclear and cytoplasmic H&E staining. Picric acid also counters the hardening effect of formalin and the swelling effect of the acetic acid.

Chromic Acid aids in fixation by oxidation and coagulating proteins.

EMS Catalog supplies mentioned	Cat. No.
Fixatives	
10% Formalin in Phosphate buffer 1L	**15740-01**
Paraformaldehyde, 4% in 0.1M Phosphate buffer 500 ml	**15735-20S**
Paraformaldehyde, 4% in 0.1M Sodium Cacodylate buffer 500 ml	**15952-15S**
Glutaraldehyde, 25% aqueous solution 10x10 ml	**16220**
Karnovsky's 3% Glutaraldehyde/ 2% Paraformaldehyde in 0.1M Phosphate buffer 1 L	**15731-10**
Osmium Tetroxide, 4% aqueous solution 10x5 ml	**19170**
100% OsO_4, crystalline 5x2 g	**19112**
Potassium Permanganate 250 g	**20200**
Potassium Ferricyanide100 g	**20150**
Tannic acid 100 g	**21700**
Uranyl Acetate 25 g	**22400**

Please check our website or catalog, as many of the above products are available in solution, in various concentrations and buffers, or in bulk granular form.

False color TEM micrograph showing a continuity between the nuclear envelope and a cistern of the RER (red). Nucleus (gold). Cytoplasm (blue). Image credit: Jose Luis Calvo



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