

## Fixation / Postfixation of samples and staining of tissue sections

### 4CF-1G Double aldehyde fixative for TEM & LM

Formaldehyde 35%	200 ml
Glutardialdehyde 25%	80 ml
NaOH	5.4 g
NaH <sub>2</sub> PO <sub>4</sub> . H <sub>2</sub> O	23.2 g
Distilled water	1720 ml

**Note:** This fixative solution must be pH 7.4 and kept at 4 °C.

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### Postfixation with Osmium Potassium Ferrocyanide

OsO <sub>4</sub> 1% in Millonig buffer	10 ml
K <sub>4</sub> Fe(CN) <sub>6</sub>	211.2 ml

**Note:** Osmium is a toxic chemical like other components of this solution. All safety measures must be taken during preparing as well as during application of this solution. Wear gloves and do not inhale toxic vapours of osmium! pH 7.4 at 4 °C.

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### Millonig Buffer Solution

Suitable vehicle for most biological materials

NaH <sub>2</sub> PO <sub>4</sub> .H <sub>2</sub> O	11.6 g
NaOH	2.7 g
Distilled water	860 ml

**Note:** This solution has a pH 7.2 and can be kept at 4 °C for 6 months.

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## Stain solutions

### Toluidine Blue 1%

Toluidine blue	1 g
Sodium tetraborate	1 g
Distilled water	100 ml

**Note 1 :** pH of this stain solution is approx. 11 and can be adjusted if needed.

Staining time : between a few second up to 1 minute according to the thickness of the sections. Decolorization of sections can be achieved using distilled or tap water. Sometimes a diluted alcohol (50%) solution may be applied.

**Note 2 :** for a Toluidine blue 0.1% stain solution simply dissolve 0.1 g stain powder in 100 ml distilled water containing 1 gm sodium tetraborax.

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### **Crystal Violet 0.1%**

Boric acid	2 g	(H <sub>3</sub> BO <sub>3</sub> )
Crystal violet	0.1 g	
Distilled water	100 ml	

Note: the rest of stain solution on the section can be washed up using tap water. Water or 50% alcohol may be used for decolorization of sections.

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### **Uranyl acetate for ultrathin sections for Transmission Electron Microscopy**

Uranyl acetate (Merck Prod. No. 8473)	6 g
Distilled water	100 ml

**Note:** Uranyl solutions are **radioactive!** Avoid any contact or inhalation! Put this solution overnight in an oven at 60 °C and then keep in dark brown glass bottle. Stain ultrathin sections at 40 °C for 10 minutes.

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