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10% Buffered Formalin vs 4% Paraformaldehyde for Tissue Preservation

Formaldehyde

Formaldehyde (HCHO) is a gas.

10% Buffered Formalin

10% formalin is a 1:10 dilution of a saturated solution of formaldehyde in water, i.e. 1 part saturated formaldehyde in water diluted with 9 parts plain water. Because the maximum concentration of formaldehyde in water is 40%, the 1:10 dilution of a saturated solution of formaldehyde in water (10% buffered formalin) would contain 4% formaldehyde. Methanol is typically added to saturated solutions of formaldehyde to inhibit polymerization.

Room temperature 10% buffered formalin should be used for preservation/fixation of tissues.

Paraformaldehyde

Paraformaldehyde is polymerized formaldehyde and is usually obtained as a white powder. If mixed with water and heated it depolymerizes and dissolves in the water. A 4% solution made by heating 4 grams paraformaldehyde in 100 ml water until it has all dissolved results in a 4% formaldehyde. Since it is made from pure formaldehyde polymer it will not contain methanol. For this reason it has been called “methanol free 10% formalin”.

Even though the chemistry is just the same, because 4% paraformaldehyde is polymerized, it does not penetrate the tissues as rapidly as 10% Buffered Formalin. Because 4% paraformaldehyde is typically refrigerated to inhibit polymerization, the chemical reactions that occur during fixation occur more slowly at the lower temperature. To achieve the same amount of cross-linking of protein molecules you might need 10 hours at 24C or 40 hours at 4C

Paraformaldehyde is appropriately used for preservation of cells in tissue culture and in some tissues for electron microscopy. Because of its inability to rapidly penetrate tissues, paraformaldehyde is **not appropriate** for fixation of tissues for histology. 10% Buffered Formalin is the correct fixative for tissues for routine paraffin processing and histology.