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TECHNICAL DATA SHEET 111

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Purified sym-Collidine Buffer

A Biologically Stable Buffer for Use with Osmium Tetroxide and Other Fixatives

Sym-Collidine has been used by Bennett and Luft¹ with hydrochloric acid as a buffering system for regulating the pH of osmium tetroxide fixing solutions for Electron Microscopy. The use of sym-Collidine in place of the traditional veronal-acetate buffer has several advantages in terms of stability and buffering capacity. The veronal-acetate system, developed by Michaelis and introduced for use in Electron Microscopy by Palade, is actually two buffer systems in one. The sodium acetate-hydrochloric acid is an effective buffer in the acid range (pH 4.2 to 6.2) and the veronal-hydrochloric acid is most efficient between pH 7.5 and 8.5. However, osmium tetroxide is most commonly used in the pH range of 7.2 to 7.5. As it can be seen, this range is not covered very efficiently by the veronal-acetate buffer. The veronal-acetate buffer cannot be stored, since it easily becomes contaminated by bacterial mold. Sym-Collidine, on the other hand, when neutralized by hydrochloric acid, is a most efficient buffer in the neighborhood of pH 7.4, where osmium tetroxide is most effective. It is almost indefinitely biologically stable when stored at room temperature and will neither react nor complex strongly with osmium tetroxide.² The use of NaOH•HIO₄ to facilitate the staining of osmium-collidine fixed sections for autoradiography has been reported.³

The addition of osmium tetroxide does not alter the pH any more than the equivalent amount of water would. Various concentrations may be used, unlike the veronal-acetate buffer, because veronal is relatively insoluble in water. Thus, isotonic or hypertonic fixing solutions can be made without recourse to other solutes.

Preparation of Buffer:

A stock solution of sym-Collidine buffer can be made by dissolving 5.34 ml of pure sym-Collidine in about 100 ml of distilled water, adding 18.0 ml of 1.0 N hydrochloric acid, and diluting to 200 ml with distilled water. This gives 200 ml of 0.2 M sym-collidine buffer with a pH of 7.40 to 7.45. This can be mixed in any proportion, with a stock solution of osmium tetroxide to give the desired concentration of fixative and buffer. The pH of the buffer rises a little upon dilution with water (see table).

Molarity of Buffer	pH of Buffer
0.20	7.44
0.10	7.49
0.05	7.50
0.025	7.50

The initial pH of the solution may also be adjusted by varying the amount of 1.0 N hydrochloric acid added to the sym-collidine. For 5.34 ml of sym-Collidine in a final volume of 200 ml, the pH of the 0.20 M buffer varied as the following table shows:

ml of 1.0 N HCl	pH of Final Stock Solution
10.0	7.74
12.0	7.67
14.0	7.59
16.0	7.50
18.0	7.41
20.0	7.33

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Specification:

Ampoules containing 5.34ml of pure sym-Collidine are available from this laboratory. The compound sold by us is rigorously purified by triple crystallization of the urea canal complex, and then careful fractionation through a 20 plate distillation column at 171°C. The sym-Collidine, which is commercially available is usually contaminated by less completely methylated pyridines, which form yellowish crystalline or oily complexes with osmium tetroxide and make it impossible to use as a buffer. The purified compound offered by us is completely clean after the addition of osmium tetroxide solution. In addition to the 5.34 ml ampoule of collidine, we supply the requisite amount of 1.0 N HCl acid to permit easy preparation of the pH buffer.

References:

1. Bennett, H.S. and Luft, J.H., J. Biophysics and Biochem. Cytol., **6**, 113 (1959).
2. Luft, J., J. Biophysics and Chem. Cytol., **2**, 779 (1956).
3. Hendrickson, A., et al., Stain Techn., **43**, 175 (1968).

Precautions:

Sym-Collidine is harmful if swallowed or inhaled and can cause eye, skin, and respiratory irritation. Hydrochloric acid is corrosive and can cause burns. Do not get either component in eyes, on skin or clothing. Avoid breathing vapors. Use with adequate ventilation. Wear protective goggles and gloves. Wash thoroughly after handling. Keep containers closed. Store away from heat, sparks and/or open flames.

Ordering Information:

Cat. #	Description	Size
00346	2,4,6-sym-Collidine Buffer Kit, E.M. Grade (2,4,6-Trimethylpyridine) Contains 6x5.34ml of Collidine and 6x18ml of 1.0 N HCl. Makes 100ml	1kit
0223A	Osmium tetroxide, crystalline 99%	5x1g
0223B	Osmium tetroxide, crystalline 99%	10x1g
0223C	Osmium tetroxide, crystalline 99%	10x1/2g
0223D	Osmium tetroxide, crystalline 99%	10x1/4g
0972A	Osmium tetroxide, 4% microfiltered solution	2x20ml amp
0972B	Osmium tetroxide, 4% microfiltered solution	5x10ml amp
0972C	Osmium tetroxide, 4% microfiltered solution	20x10ml amp

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