

## Laboratory for Advanced Electron and Light Optical Methods

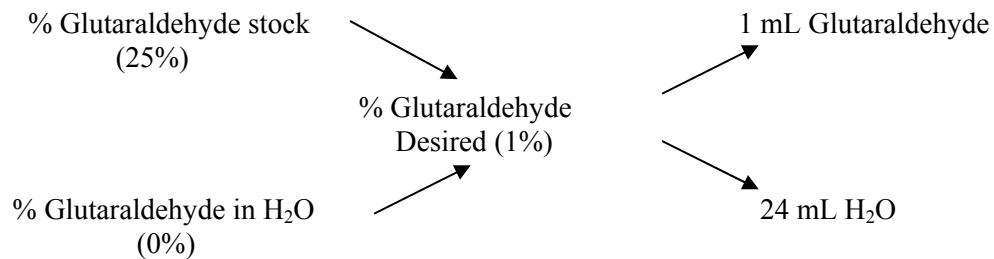
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### Preparation of Buffers

#### I. Preparing Dilutions of Solutions

A. **Purpose:** The following example is given to help explain how to take a stock of a given concentration and to produce a solution of lesser concentration.

B. **Procedure:**



#### II. Sorenson's Sodium Phosphate Buffer

A. **Preparation of Phosphate Buffer at pH 7.2-7.4 pH Used for Normal TEM and SEM Sample Preparation:**

1. Stock solutions:

a. **Stock A:** 0.2 M solution of NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O

Add 27.6 g of NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O to 900 ml of distilled water and bring the final volume to 1 L

b. **Stock B:** 0.2 M solution of Na<sub>2</sub>HPO<sub>4</sub> (anhydrous)

Add 28.4 g of Na<sub>2</sub>HPO<sub>4</sub> (anhydrous) to 900 ml of distilled water and bring the final volume to 1 L

2. Preparation of **Mixing Buffer** (0.2 M buffer) for mixing with fixatives:

Mix 23 ml of **stock A** and 77 ml of **stock B**

The pH of the final solution should be 7.2-7.4, but it should be checked

a pH meter to be sure. The pH may be adjusted using 0.1 N HCl to lower it or 0.1 N NaOH to raise it. Try not to overshoot the proper pH.

3. **Preparation of 0.1 M Washing Buffer solution for washes**

- a. Mix 23 ml of **stock A** and 77 ml of **stock B** and add to 100 ml of distilled water. **Check to make sure that the pH is 7.2-7.4 and adjust as suggested above.**
- b. Alternatively, equal volumes of previously prepared 0.2 M **Mixing Buffer** and distilled water can be combined to make a 0.1 M **Washing Buffer**.

B. **Preparation of Sorenson's Sodium Phosphate Buffer at Various pHs:**

1. Preparation of **Stock Solutions A and B** as described above.
2. Preparation of 0.2 M **Working Buffer** of different pHs:

mL of Stock A	mL of Stock B	Final pH of Solution
68.5	31.5	6.5
62.5	37.5	6.6
56.5	43.5	6.7
51.0	49.0	6.8
45.0	55.0	6.9
39.0	61.0	7.0
33.0	67.0	7.1
28.0	72.0	7.2
23.0	77.0	7.3
19.0	81.0	7.4
16.0	84.0	7.5

**Reference:** Sorenson, *Biochem. A.*, 22:352 (1909). Cited by Gomori, G., in *Methods in Enzymology*, S.P. Colowick and N.O. Caplan (eds.). Volume 1:142-143 (1965). Academic Press, NY.

III. **Preparation of 0.2 M Sodium Cacodylate Buffer, pH 7.2-7.4:**

Add 42.8 g of  $\text{Na}(\text{CH}_3)_2\text{AsO}_2 \cdot 3\text{H}_2\text{O}$  to 900 ml of distilled water and bring the final volume to 1 L

**Or**

Add 31.99 g of  $\text{Na}(\text{CH}_3)_2\text{AsO}_2$  to 900 ml of distilled water and bring the final volume to 1 L

**Adjust the pH with 0.1 N HCl**

**Use caution when working with the solid sodium cacodylate and the buffer solutions: The compounds are toxic and contain arsenic.**

**IV. Preparation of Dulbecco's Phosphate-Buffered Saline (PBS):**

Add the following materials to 900 ml of distilled water:

0.2 g KCl

0.2 g  $\text{KH}_2\text{PO}_4$

8.0 g NaCl

1.15 g  $\text{Na}_2\text{HPO}_4$

**Bring the final volume up to 1 L and adjust the pH to 7.2-7.4 as described for Sorenson's sodium phosphate buffer above.**