

phosphate buffer

Information from cshprotocols.org:

Gomori buffers, the most commonly used phosphate buffers, consist of a mixture of monobasic dihydrogen phosphate and dibasic monohydrogen phosphate. By varying the amount of each salt, a range of buffers can be prepared that buffer well between pH 5.8 and pH 8.0 (please see the tables below). Phosphates have a very high buffering capacity and are highly soluble in water. However, they have a number of potential disadvantages:

* Phosphates inhibit many enzymatic reactions and procedures that are the foundation of molecular cloning, including cleavage of DNA by many restriction enzymes, ligation of DNA, and bacterial transformation.

* Because phosphates precipitate in ethanol, it is not possible to precipitate DNA and RNA from buffers that contain significant quantities of phosphate ions.

* Phosphates sequester divalent cations such as Ca²⁺ and Mg²⁺.

0.5L of 1M K₂HPO₄ at 174.18g mol⁻¹ = 87.09g

0.5L of 1M KH₂PO₄ at 136.09g mol⁻¹ = 68.045g

preparation of 0.1 M potassium phosphate buffer at 25°C

Preparation of 0.1 M Potassium Phosphate Buffer at 25°C

pH	VOLUME OF 1 M K ₂ HPO ₄ (ml)	VOLUME OF 1 M KH ₂ PO ₄ (ml)
5.8	8.5	91.5
6.0	13.2	86.8
6.2	19.2	80.8
6.4	27.8	72.2
6.6	38.1	61.9
6.8	49.7	50.3
7.0	61.5	38.5
7.2	71.7	28.3
7.4	80.2	19.8
7.6	86.6	13.4
7.8	90.8	9.2
8.0	94.0	6.0

Dilute the combined 1 M stock solutions to 1 liter with distilled H₂O. pH is calculated according to the Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK}' + \log \left\{ \frac{(\text{proton acceptor})}{(\text{proton donor})} \right\}$$

where pK' = 6.86 at 25°C.