

HEPES (Free Acid)

A buffer is most effective when its pH is equal its pKa, and is therefore most efficient when in the range of one pH unit above and below that value. The pKa of sodium bicarbonate, for example, is 6.1, yielding the best buffering capacity at a pH of 5.1-7.1. When higher buffer pH values are needed, or maintain the pH, HEPES is often added.

The organic zwitterionic buffer HEPES is commonly used to maintain pH levels of basal media in cell culture. In comparison to the inorganic sodium bicarbonate buffer system, HEPES is more suitable for buffering in the physiological pH range of 7.2-7.6.

HEPES contains both positive and negative ionizable groups, where the secondary and tertiary amine groups provide the positive charge and the negative charges are offered by the sulfonic and carboxylic acid groups.¹

Unlike the bicarbonate buffering system that requires the set of a CO₂ incubator, the HEPES buffering system may be used with or without a CO₂ atmosphere. When HEPES is used with exogenous CO₂, however, the concentration of HEPES must be more than double the amount of bicarbonate for adequate buffering.²

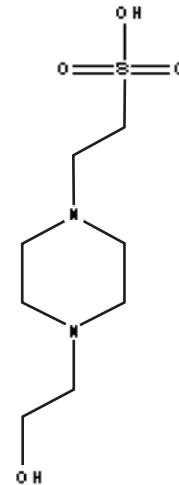
Synonym:

N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid

Molecular Weight and Formula:

238.31, C₈H₁₈N₂O₄S

Molecular Structure:



Appearance:

Fine white powder or clear colorless solution

Working Concentration:

HEPES may be added to media at concentrations of 10mM-25mM. Concentrations of 50mM and above are not recommended and may be toxic to cells.

Storage:

Powder: Ambient (15-30°C); protected from light
Liquid: Ambient (15-30°C); protected from light

Product Specifications: Liquid

pH	7.2 ± 0.2 at 20-25°C
Osmolality	1650 ± 100 mOsm/kg H ₂ O
Endotoxin	≤ 0.25 EU/mL at 1:100
Mycoplasma	Tested negative
Sterility by USP	Pass

Product Description	Catalog No.	Size
HEPES (Free Acid) 1M Solution, Liquid	25-060-CI	6 x 100 mL
HEPES (Free Acid) Powder	61-034-RM 61-034-RO 61-034-RR	1 x 100 g 1 x 500 g 1 x 1 kg

References:

- Good et al. 1966. Hydrogen ion buffers for biological research. *Biochemistry* 5:467-477
- Freshney R Ian. 2000. *Culture of Animal Cells: A Manual of Basic Technique*. 4th ed. New York (NY): Wiley-Liss. p. 90-91