

Product Information

HEPES FREE ACID

**Sigma Prod. Nos. H3375, H7523,
H6147, H9136 and H4034**

CAS NUMBER: 7365-45-9

SYNONYMS: N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

PHYSICAL DESCRIPTION:

Appearance: White powder¹

Molecular formula: C₈H₁₈N₂O₄S

Molecular weight: 238.3

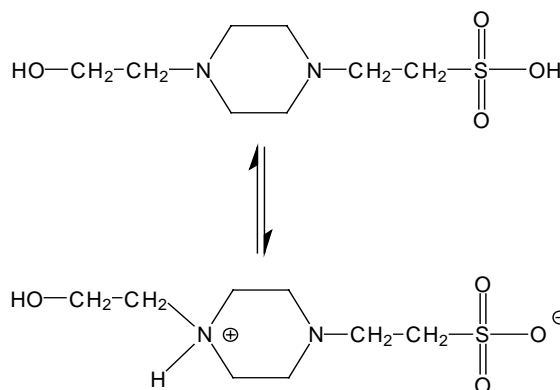
pK_{a1}: ≈ 3^{1,3}

pK_{a2}: 7.85 at 0°C^{1,3}

7.55 at 20°C^{1,3}

7.31 at 37°C^{1,3}

ΔpK/ΔT = -0.014/°C⁴



FOR SPECIFICATIONS SEE CATALOG

HEPES does not bind magnesium, calcium, manganese(II) or copper(II) ion.⁵

SOLUBILITY / SOLUTION STABILITY:

A 1 M solution (954 mg in 4 mL water) 1639 mOsm/kg is clear and colorless, with pH approximately between 5.0 and 6.5 at 20°C.¹ At 0°C, a saturated solution is reportedly 2.25 M.² Solutions may be autoclaved under standard conditions.^{1,3}

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GENERAL USAGE:

HEPES has been described as one of the best all-purpose buffers available for biological research.⁴ At most biological pHs the molecule is zwitterionic, and is effective as a buffer at pH 6.8 to 8.2. HEPES has been used in a wide variety of applications, including tissue culture.

Buffer strength for cell culture applications is usually in the range of 10 to 25 mM; the Sigma general catalog has data supporting the use of HEPES in media formulations to stabilize pH at 37°C.⁶ After the addition of HEPES pH is adjusted with NaOH or HCl. Care must be taken to maintain appropriate osmolality in media, and toxicity with respect to a given cell line must be evaluated. (Isotonicity data have been tabulated.⁷) HEPES is reportedly superior to NaHCO₃ in controlling pH in tissue and organ culture.⁸

HEPES is not recommended for certain protein applications; it interferes with the Folin-Ciocalteu protein assay. The Biuret protein assay is unaffected.⁹

HEPES was the buffer of choice in a protein deposition technique in electron microscopy because it did not affect metal substrates.¹¹ HEPES was evaluated and shown to be quite suitable for use with Ampholines in generating pH gradients less than 1 pH unit wide for isoelectric focusing applications.¹²

A buffer solution of HEPES can be prepared by any of several methods. The free acid can be added to water, then titrated with approximately one-half mole equivalent of sodium hydroxide or potassium hydroxide to the pH desired, a simple mixing table for preparing 0.05 M HEPES/NaOH has been published.¹³ Alternatively, equimolar concentrations of HEPES and of sodium HEPES can be mixed in approximately equal volumes, back-titrating with either solution to the appropriate pH.

For convenient buffer preparation, Sigma offers a number of related products: HEPES free acid (H3375), potassium HEPES (H0527), sodium HEPES (H8651, H7006, H2393), and hemisodium HEPES in bulk (H7637) and as "instant buffer" foil pouches (H9897). H7523, HEPES free acid, Sigma Ultra, is tested for trace metals; H6147, Embryo Tested; H9136, Cell Culture Tested; and H4034, Biotechnology Performance Certified.

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CITED REFERENCES:

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