ProductInformation

HEPES SODIUM SALT, HYDRATE
Sigma Prod. No. H2393

CAS NUMBER: 75277-39-3
SYNONYMS: sodium N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonate); sodium 4-(2-hydroxyethyl)-1-piperazineethanesulfonate

PHYSICAL DESCRIPTION:

Appearance: White powder[1]
Molecular formula:
$C_8H_{17}N_2O_4SNa$
Molecular weight: 260.3
pKa_1: ≈ 3[1,3]
pKa_2: 7.85 at 0°C[1,3]
7.55 at 20°C[1,3]
7.31 at 37°C[1,3]
$\Delta pK/\Delta T$ = -0.014/°C[4]

FOR SPECIFICATIONS
SEE CATALOG

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

STABILITY / STORAGE AS SUPPLIED:

HEPES sodium salt is stable at least three years if stored sealed and kept dry at room temperature. Although Sigma does not assign expiration dates, sodium HEPES should be re-evaluated for continued suitability in user application every three to five years.

SOLUBILITY / SOLUTION STABILITY:

A solution of 25 g in 50 mL water (33% w/w) is clear, and colorless to very faint yellow, with pH approximately 10.5 at room temperature.[1] At 0°C, a saturated solution of the free acid 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 most 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] 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 $NaHCO_3$ in controlling pH in tissue and organ culture.[8]

Unfortunately, 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 precise pH desired, with adjustments made for final temperature and volume. (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. Titrating H2393 with hydrochloric acid will yield a buffer solution containing a half-equivalent of sodium chloride; this much additional ionic strength will significantly change the osmolality of the solution.

For convenient buffer preparation, Sigma offers a variety of related products: HEPES (H3375) and HEPES SigmaUltra (H7523), potassium HEPES (H0527), sodium HEPES (H8651, H7006), and hemisodium HEPES in bulk (H7637) and as "instant buffer" foil pouches (H9897). See also the application-tested products in the Molecular Biology and Cell Culture sections of the catalog.

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CITED REFERENCES:

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