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: \(C_9H_{17}N_2O_4SNa\)  
Molecular weight: 260.3  
pKa\(_1\): \(=3\)^\(^1,3\)  
pKa\(_2\): 7.85 at 0\(^\circ\)C\(^1,3\)  
7.55 at 20\(^\circ\)C\(^1,3\)  
7.31 at 37\(^\circ\)C\(^1,3\)  
\(\Delta pK/\Delta T = -0.014/\circ\)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\(^\circ\)C, a saturated solution of the free acid is reportedly 2.25 M.\(^2\) Solutions may be autoclaved under standard conditions.\(^1,3\)
GENERAL USAGE:

HEPES has been described as one of the best all-purpose buffers available for biological research. 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. 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.) HEPES is reportedly superior to NaHCO₃ in controlling pH in tissue and organ culture.

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

HEPES was the buffer of choice in a protein deposition technique in electron microscopy because it did not affect metal substrates. 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.

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.) 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:

1. Sigma quality control.
6. Sigma Cell Culture.