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## Product Information

### HEPES sodium salt

Product Number **H 3784**  
Store at Room Temperature

#### Product Description

Molecular Formula:  $C_8H_{17}N_2NaO_4S$

Molecular Weight: 260.3

CAS Number: 75277-39-3

$pK_{a1}^2 = 3$

$pK_{a2} = 7.85 (0\text{ }^\circ\text{C}); 7.55 (20\text{ }^\circ\text{C}); 7.31 (37\text{ }^\circ\text{C})^2$

$\Delta pK/\Delta T^3 = -0.014/^\circ\text{C}$

Synonyms: sodium N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonate); sodium 4-(2-hydroxyethyl)-1-piperazineethanesulfonate

This product is designated as Biotechnology Performance Certified (BPC) reagent grade and meets the specifications of the USP, EP, and JP for reagent chemicals. In addition, this product can be used for molecular biology, cell culture, and electrophoresis applications.

HEPES does not bind magnesium, calcium, manganese(II), or copper(II) ions.<sup>4</sup>

HEPES has been described as one of the best all-purpose buffers available for biological research.<sup>3</sup> At most biological pHs, the molecule is zwitterionic, and is most effective as a buffer at pH 6.8 to 8.2 ( $pK \pm 1$ , as a general rule). 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. 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.<sup>5</sup> HEPES is reportedly superior to  $NaHCO_3$  in controlling pH in tissue and organ culture.<sup>6</sup>

Unfortunately, HEPES is not recommended for certain protein applications. It interferes with the Folin-Ciocalteu protein assay. The Biuret protein assay is unaffected.<sup>7</sup>

HEPES was the buffer of choice in a protein deposition technique in electron microscopy because it did not affect metal substrates.<sup>9</sup> HEPES was evaluated and shown to be quite suitable for use with Ampholines<sup>®</sup> in generating pH gradients less than 1 pH unit wide for isoelectric focusing applications.<sup>10</sup>

#### Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

#### Preparation Instructions

A solution of 25 g in 50 ml water (33% w/w) is clear and colorless, with pH approximately 10.5 at room temperature.<sup>1</sup>

#### Storage/Stability

Solutions may be autoclaved under standard conditions.<sup>2</sup>

#### Procedure

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 H 3784 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. A buffer solution of HEPES can be prepared by 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.<sup>11</sup> 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 H 3784 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.

## References

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JSX/RXR 7/03

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