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## Product Information

### HEPES SODIUM SALT

Sigma Prod. No. H7006

Store at Room Temperature

**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<sup>1</sup>

Molecular formula: C<sub>8</sub>H<sub>17</sub>N<sub>2</sub>O<sub>4</sub>SNa

Molecular weight: 260.3

pK<sub>a1</sub>: .3<sup>1,3</sup>

pK<sub>a2</sub>: 7.85 at 0°C<sup>1,3</sup>

7.55 at 20°C<sup>1,3</sup>

7.31 at 37°C<sup>1,3</sup>

ΔpK/ΔT = -0.014/°C<sup>4</sup>

### FOR SPECIFICATIONS SEE CATALOG

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

### SOLUBILITY / SOLUTION STABILITY:

A solution of 25 g in 50 mL water (33% w/w) has a pH of approximately 10.5 at room temperature.<sup>1</sup> At 0°C, a saturated solution of the free acid is reportedly 2.25 M.<sup>2</sup> Solutions may be autoclaved under standard conditions.<sup>1,3</sup>

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**GENERAL USAGE:**

HEPES has been described as one of the best all-purpose buffers available for biological research.<sup>4</sup> At most biological pHs the molecule is zwitterionic, and is most effective as a buffer at pH 6.8 to 8.2 ( $pK \approx 1$ , 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; the Sigma general catalog has data supporting the use of HEPES in media formulations to stabilize pH at 37°C.<sup>6</sup> 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>7</sup>) HEPES is reportedly superior to NaHCO<sub>3</sub> in controlling pH in tissue and organ culture.<sup>8</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>9</sup>

HEPES was the buffer of choice in a protein deposition technique in electron microscopy because it did not affect metal substrates.<sup>11</sup> 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.<sup>12</sup>

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.<sup>13</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 H7006 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, H2393), 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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