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## Product Information

### HEPES solution Cell Culture Tested

Product Number **H 0887**  
Storage Temperature 2-8 °C

#### Product Description

Molecular Formula:  $C_8H_{18}N_2O_4S$  (HEPES solid, free acid)

Molecular Weight: 238.3 (HEPES solid, free acid)

CAS Number: 7365-45-9

Synonym: 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid solution

This product is a  $1.0 \pm 0.1$  M solution. It is prepared from HEPES free acid and cell culture grade water. The pH has been adjusted to  $7.3 \pm 0.3$ . This product is aseptically filled, cell culture tested (25 ml/L), and tested for endotoxin levels.

HEPES is a zwitterionic buffer that is widely used in biochemistry and molecular biology research. It is one of the Good buffers developed in the 1960's to provide buffers in the pH range of 6.15 - 8.35 for wide applicability to biochemical studies. The pioneering publication by Good and co-workers describes the synthesis of HEPES and its physical properties.<sup>1</sup> The useful pH range of HEPES is 6.8 - 8.2.

In cell culture research, HEPES is utilized in buffering Dulbecco's Modified Eagle Medium (DMEM, 10 mM, pH 7.15) and saline (50 mM, pH 7.05).<sup>2</sup> HEPES is reportedly superior to  $NaHCO_3$  in controlling pH in tissue and organ cultures.<sup>3</sup> HEPES has been utilized in a study of internal pH regulatory mechanisms in cultured rat cerebellar granule cells.<sup>4</sup> The effect of HEPES buffered systems on the growth of *Mycoplasma mycoides subsp. Mycoides* small colony vaccine cultures has been investigated.<sup>5</sup> HEPES buffered media from pH 7.0 - 7.8 have been utilized to study the activity of cultured human osteoblasts.<sup>6</sup>

Protocols have been described for the use of HEPES in buffers for identifying DNA-binding proteins in bacteriophage  $\lambda$  expression libraries, cell resuspension, DNase I dilution, electrophoresis, oligonucleotide labeling, random primers, and tissue

homogenization and resuspension.<sup>2</sup> In protein assay studies, HEPES has been reported to interfere with the Folin-Ciocalteu protein assay, while the Biuret protein assay is unaffected.<sup>7</sup> A protocol for casein zymography of calpains that utilizes a HEPES/imidazole buffer has been reported.<sup>8</sup> The use of HEPES in the analysis of histone H1 and other basic proteins by cationic disc electrophoresis in polyacrylamide gels has been described.<sup>9</sup>

#### Precautions and Disclaimer

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

#### References

1. Good, N. E., et al, Hydrogen ion buffers for biological research. *Biochemistry*, **5(2)**, 467-477 (1966).
2. Molecular Cloning: A Laboratory Manual, 3rd ed., Sambrook, J. and Russell, D. W., CSHL Press (Cold Spring Harbor, NY: 2001), pp. 14.33, 14.36, 17.6, 17.19, 13.56, 9.10, 9.6, 9.47, 17.6, 17.25, 16.32, 16.15-16.17, 16.22-16.23, 16.52.
3. Shipman, C., in *Tissue Culture, Methods and Applications*, Kruse Jr., P. F., and Patterson Jr., M. K., eds., Academic Press (New York, NY: 1973), p. 709.
4. Pocock, G., and Richards, C. D., Hydrogen ion regulation in rat cerebellar granule cells studied by single-cell fluorescence microscopy. *Eur. J. Neurosci.*, **4(2)**, 136-143 (1992).
5. Waite, E. R., and March, J. B., Effect of HEPES buffer systems upon the pH, growth and survival of *Mycoplasma mycoides subsp. Mycoides* small colony (MmmSC) vaccine cultures. *FEMS Microbiol. Lett.*, **201(2)**, 291-294 (2001).
6. Kaysinger, K. K., and Ramp, W. K., Extracellular pH modulates the activity of cultured human osteoblasts. *J. Cell. Biochem.*, **68(1)**, 83-89 (1998).

7. Himmel, H. M. and Heller, W., Studies on the interference of selected substances with two modifications of the Lowry protein determination. *J. Clin. Chem. Clin. Biochem.*, **25(12)**, 909-913 (1987).
8. Croall, D. E., et al., Casein zymography of calpains using a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-imidazole buffer. *Anal. Biochem.*, **304(1)**, 129-132 (2002).
9. Paulson, J. R., et al., Rapid analysis of mitotic histone H1 phosphorylation by cationic disc electrophoresis at neutral pH in minigels. *Anal. Biochem.*, **203(2)**, 227-234 (1992).

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