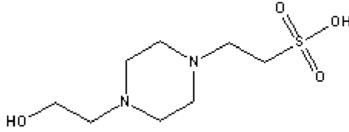
Catalog Number: 101926, 105593, 152451, 1588413, 1588415, 1588416, 1588417, 1688446, 1688449, 194549, 194550, 194827, 194828

HEPES

Structure (free acid):



	Molecular Formula	Molecular Weight	CAS #
Free Acid	$C_8H_{18}N_2O_4S$	238.3	7365-45-9
Hemisodium	$C_8H_{17}N_2O_4S \cdot ½Na$	249.3	103404-87-1
Sodium Salt	$C_8H_{17}N_2O_4SNa$	260.3	75277-39-3

Synonyms: N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid); 4-(2-Hydroxyethyl)-1- piperazineethanesulfonic acid

pKa's:[2]

pKa_1: ~3
pKa_2 @ 0°C: 7.85
pKa_2 @ 20°C: 7.55
pKa_2 @ 37°C: 7.31
$\Delta pK/\Delta T$: -0.014/°C[3] *Note:* since the ΔpKa/°C of -0.014, the pH reading recorded in a HEPES buffered medium will vary inversely with the temperature of the medium.

Physical Description: White crystalline powder

Useful pH Range: 6.8 to 8.2

The following table is expected pH levels at various temperatures:

Temperature °C	pH	Temperature °C	pH
5	7.58	23	7.47
15	7.56	24	7.46
16	7.55	25	7.44
17	7.54	26	7.43
18	7.53	27	7.42
19	7.52	28	7.41
20	7.5	29	7.4
21	7.44	30	7.38
22	7.48	37	7.3

Buffer Type: Zwitterionic buffer

Solubility: A 1 M solution is clear and colorless. At 0°C, a saturated solution of the free acid is reportedly 2.25 M.[1] Soluble at 1% solution in water at 25°C. Solutions may be autoclaved under standard conditions.[2] HEPES is light sensitive when added to media. Solutions should be stored in the dark to avoid possible toxic effects.

	pH of a 1 M Solution at 20°C
Free Acid	approximately between 5.0 and 6.5 (1639 mOsm/kg)
Sodium Salt	approximately between 10.0 and 12.0
Hemisodium Salt	approximately 7.5

Formulation (for #16884):

Component	***mg/liter***	***Mol. Wt.***	***Mol. (mM)***
HEPES	238300.00	238.3	1000.00

Description: HEPES is a general-purpose zwitterionic buffer which does not bind magnesium, calcium, manganese(II) or copper (II)

ions.[4]

Buffer strength for cell culture applications is usually in the range of 10 to 25 mM. After the addition of HEPES, the pH is adjusted with NaOH or HCl. Care must be taken to maintain the appropriate osmolality in media, and toxicity with respect to a given cell line must be evaluated (Isotonicity data have been tabulated.[5]). HEPES is reportedly superior to sodium bicarbonate in controlling the pH in tissue and organ cultures.[6]

HEPES may exhibit toxicity at concentrations greater than 40 mM. Studies have indicated that 20 mM HEPES is the most satisfactory concentration of the buffer when both Hanks' and Earle's solutions are used. CO_2 incubators should not be used with media buffered solely with HEPES.

HEPES is not recommended for certain protein applications such as the Folin-Ciocalteu protein assay; however, it does not affect the Biuret protein assay.[7]

HEPES is the buffer of choice in a protein deposition technique in electron microscopy because it did not affect metal substrates.[9] HEPES has been evaluated and shown to be suitable for use with Ampholines in generating pH gradients less than 1 pH unit wide for isolectric focusing applications.[10]

HEPES is the recommended buffer for the glutamate binding assay because it prevents binding to non-receptor materials.[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 desired pH. A simple mixing table for preparing 0.05 M HEPES/NaOH has been published.[11] Alternatively, equimolar concentrations of HEPES and of HEPES sodium salt can be mixed in approximately equal volumes and back-titrate with either solution to the appropriate pH. The sodium salt can be titrated with HCl to yield a half-equivalent of sodium chloride; however, the addition of the ionic strength will change the osmolality of the solution. Most solutions of HEPES hemisodium will disolve in water forming a pH at 7.5 with little to no adjustment.

Typical Formulations for HEPES buffered Saline (2X):

Formula #1[13]**:**

Sodium Chloride	1.6 g
Potassium Chloride	0.074 g
Na_2HPO_4 dihydrate	0.027 g
Dextrose	0.2 g
HEPES, free acid	1 g

Dissolve above ingredients in a total volume of 90 ml of distilled water. Adjust the pH to 7.05 with 0.5 N NaOH, and then adjust the volume to 100 ml with distilled water. Sterilize the solution by passage through a 0.22 micron filter. Store in aliquots at -20°C.

Formula #2[14]**:**

Sodium Chloride	16.4 g
HEPES, free acid	11.9 g
Na_2HPO_4	0.21 g
Water	to 1 liter

Add the above ingredients and stir until dissolved. Titrate to pH 7.05 with 5 M NaOH (an exact pH is extremely important). Filter sterilize.

Test the formulation before use with transfection experiments. Test by mixing 0.5 ml of the above buffer with 0.5 ml 250 mM $CaCl_2$ and vortexing. A fine precipitate should develop that is readily visible in the microscope. If this precipitate does not form, do not use the batch of buffer for transfection experiments.

Availability:

Catalog Number	Description	Size
101926	HEPES, free acid, purity approximately 99%	25 g 50 g 100 g 250 g 500 g 1 kg 5 kg

194549	HEPES, free acid, cell culture reagent	10 g 25 g 50 g 100 g 250 g 500 g 1 kg
194827	HEPES, free acid, molecular biology reagent	25 g 100 g 500 g
152451	HEPES, Hemisodium salt, 0.5 mole sodium per mole HEPES	25 g 100 g
105593	HEPES, Sodium salt, purity approximately 99%	25 g 100 g 250 g 1 kg
194550	HEPES, Sodium salt, cell culture reagent	10 g 25 g 100 g 250 g 1 kg
194828	HEPES, Sodium salt, molecular biology reagent	25 g 100 g 500 g
1688446 1688449	HEPES Buffer, 1 M Solution, Cell culture grade; pH 7.2 to 7.4 at 37°C	20 ml 100 ml
1588413 1588415 1588416 1588417	HEPES powder, cell culture grade	20 g 50 g 100 g 500 g

References:

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