National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Materials

2181 HEPES and 2182 HEPES Sodium Salt

Standard Reference Materials (SRMs), 2181-HEPES (N-2-Hydroxyethyl-piperazine-N'-2-ethanesulfonic acid) and 2182-HEPES Sodium Salt (NaHEPESate) are intended for preparing solutions for use in calibrating clinical instruments for blood pH measurements in the physiologically important range of pH 7 to 8.

The pH(s) values correspond to log $(1/a_H)$, where a_H is the conventional activity of the hydrogen ion referred to the standard state (p^o=1 atmosphere) on the molal (mol/kg) scale. The certified values listed below were derived from emf measurements of cells without liquid junction. [1] The uncertainties of the assigned values of pH(s) for the 0.05 mol/kg HEPES buffer and for the 0.08 mol/kg HEPES and NaCl buffer are not expected to exceed ± 0.01 and ± 0.015 units, respectively, for the temperature range of 0 to 50 °C.

The pH(s) values for a buffer solution 0.05 mol/kg with respect to HEPES and NaHEPESate (HEPES-NaHEPESate) as a function of temperature are:

°C	pH(s)	°C	pH(s)	°C	pH(s)
0	7.832	20	7.565	37	7.364
5	7.763	25	7.503	40	7.327
10	7.696	30	7.443	45	7.271
15	7.630	35	7.384	50	7.216

The pH(s) values for a buffer solution 0.08 mol/kg with respect to HEPES, NaHEPESate, and NaCl (HEPES-NaHEPESate-NaCl) as a function of temperature are:

°C	pH(s)	°C	pH(s)	°C	pH(s)
0	7.853	20	7.579	37	7.373
5	7.782	25	7.516	40	7.335
10	7.714	30	7.454	45	7.278
15	7.646	35	7.394	50	7.222

The HEPES and HEPES Sodium Salt were obtained from the Sigma Chemical Co., St. Louis, Missouri.

The analytical measurements for material homogeneity and hydrogen ion activity were performed by Y.C. Wu and W.F. Koch of the NIST Inorganic Analytical Research Division, and D. Feng, Guest Scientist, People's Republic of China.

The technical and support aspects involved in the original preparation, certification, and issuance of these Standard Reference Materials were coordinated through the Standard Reference Materials Program by R.W. Seward. Revision of this certificate was coordinated through the Standard Reference Materials Program by J.C. Colbert.

Gaithersburg, MD 20899
March 4, 1992
(Revision of Certificate dated 3-17-89)

William P. Reed, Chief
Standard Reference Materials Program

(over)

DIRECTIONS FOR USE

These SRMs should be dried in a vacuum oven at 2 to 3 Pa and 50 °C for 24 h. Reagent grade NaCl should be dried in an oven at 110 °C for 12 h. All three materials should then be stored in a desiccator over magnesium perchlorate.

Preparation of the 0.05 mol/kg HEPES-NaHEPESate buffer solution:
Transfer 11.916 g HEPES and 13.015 g NaHEPESate to 1000.0 g of distilled water and mix thoroughly.

Preparation of the 0.08 mol/kg HEPES-NaHEPESate-NaCl buffer solution:
Transfer 19.065 g HEPES, 20.823 g NaHEPESate, and 4.676 g NaCl, respectively to 1000.0 g of distilled water and mix thoroughly.

The distilled water should not have a conductivity greater than 2×10^{-6} S/cm.

The water used in the preparation of these pH buffer solutions need not be protected from atmospheric carbon dioxide, nor are elaborate precautions needed to exclude air from the solutions. The solutions should, however, be protected against evaporation and from contamination by molds. For highest accuracy, buffer solutions should be replaced monthly or whenever mold is detected.

REFERENCE

[1] Feng, D., Koch, W.F., Wu, Y.C. The Second Dissociation Constant and pH of N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) from 0 to 50 °C, Anal. Chem. 61, 1400, (1989).