Technical Data Sheet

HEPES

for cell biology

Order number: 1194

HEPES or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid is a zwitterionic organic buffering agent with a pKa of 7.3 (at 37 °C). Its buffering capacity is therefore greatest in the range between pH 6.8 and pH 7.8. HEPES is readily soluble in water (~40 g/100 ml), membrane impermeable and (bio)chemically stable.
HEPES is therefore an important biological buffer widely used to stabilize the pH in enzyme reactions and media for cell and tissue culture.

Application

HEPES for cell biology is intended as a pH stabilizer in cell and tissue culture media - together with, or as a substitute for bicarbonate.
Despite its low pKa of 6.15 (at 37 °C), the bicarbonate (sodium hydrogen carbonate) - CO_2 system is the most commonly used buffer system for cell culture media because it corresponds to the body's physiological buffer system and bicarbonate ions play a crucial role in maintaining intracellular pH.
The disadvantage of bicarbonate as a sole buffer system is that it is very sensitive to changes in the ambient CO_2 concentration. Thus, if the CO_2 incubator is opened regularly, or if cells are manipulated outside the incubator for extended periods of time, it can become critical. Here, the addition of HEPES is recommended to ensure stable buffering of the cell culture medium at a pH of 7.2 to 7.6.
The level of HEPES in cell culture media may vary from 10mM to 25mM with 25mM being the most common HEPES concentration.
Caution: Cell toxicity may occur at concentrations above 100 mM. To prevent oxidation, store HEPES-containing solutions protected from light.

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