Product Name

Name: HEPES Buffer Solution (1 M)

Cat. No.: C3544-0100

Size: 100 mL

Product Description

When considering biological pH, the HEPES molecule is zwitterionic, and it is a buffer with the capability of carrying a positive charge at one end of the molecule and a negative charge at the other. It has been described as one of the best all-around, multi-purpose buffers available for biological research. It is a commonly used buffer ideal for most cell culture work as it helps to maintain pH levels, especially in basal media in culture. This is mainly due to its ability to maintain the physiological pH despite the changes in CO_2 concentration which occurs normally in cell culture due to cellular respiration. When compared to bicarbonate buffers also commonly used in cell culture, it is claimed that HEPES may not only be a more effective buffering reagent for maintaining enzymes' structure and function at lower temperatures but also is reportedly superior to $NaHCO_3$ in controlling pH in tissue or organ cultures. pH buffering is deemed necessary not only due to the fact that the growth of many cells is restricted to be within a narrow pH limit but also because cellular metabolism frequently alters the pH of the surrounding of the cells. However, the choice of buffers is dependent upon many factors including optimal milieu conditions, nutrient niche requirements, specific cell lines, general circumstances, and most of all, the researcher's experience. Buffer strength for cell culture applications is usually within the range of 10 - 25 mM. Diligence and care must be exercised to the utmost to maintain an appropriate osmolality in the media for a specific cell line.

HEPES provides the best buffer range within the pH of 6.15 - 8.35 showing its wide applicability and its most efficacious nature (i.e., *pKa* +/- 1, as a general rule). Although the most commonly used buffering system is bicarbonate due to its nutritional benefits in spite of its reduced buffering capacity at physiological pH, the supplementation of HEPES provides an additional advantage to cell culture medium within a pH range that is commensurate with the concentration utilized. Although HEPES offers no nutritional benefit to cells, it is supplemented solely for its extra buffering capacity when the cell culture may require manipulation for extended periods outside the CO_2 incubator. Biological buffers are not only utilized in *in vitro* cell culture but also in enzyme assays and some electrophoretic applications at physiological pH. Universally applicable buffers for biochemical applications must have some of the following characteristics:

- Must be H_2O-soluble
- Show non-interference with biological processes or biological membranes in terms of (e.g., surface adsorption, solubilization, penetration)
- Have known complex-forming tendency with metal ions
- Are non-toxic
- Have a Low UV absorption wavelength

All these biological processes are pH sensitive and therefore dependent on a very narrow pH range for optimal physiological metabolic equilibrium and stability by getting rid of or neutralizing these acids effectively. The purpose of a buffer in any biological system is to help maintain a normal equilibrium of the

extracellular and intracellular pH within the narrowest of ranges that resist superficial changes in the pH level in the presence of external and internal influences. Most cells require a pH condition within 7.2 - 7.4 and controlling the pH is a key, essential factor for creating the most optimum cell culture milieu. There are major variations to this optimum, as the ideal pH at culture initiation should be nearer to 7.4, but should not fall below 7.0 during the culture. A pH below 6.8 usually inhibits cell growth. Continuous transformed cell lines (e.g., hybridoma cell lines) prefer more of an acidophilic milieu (7.0 - 7.4). Thus, the regulation of pH is critical immediately following cell seeding when the new culture is acclimatizing itself to its new environment. This is usually achieved by one of two buffering systems:

The normal buffer system from the cell culture media constituents analogous to the blood in which the gaseous CO_2 stabilizes with the HCO_3^- / CO_3^{2-} content.

Improved buffering and pH stability using a zwitterion buffer such as HEPES either in addition to or instead of bicarbonate

Generally, culture media are buffered to compensate for the cellular production of CO_2 and lactic acid as the by-products of cellular metabolism. The traditional role of buffering culture media is with bicarbonate (HCO_3) ions. As cells grow and generate CO_2 continuously, an accumulation of CO_2 in the headspace will prevent CO_2 from diffusing out of the medium with a resultant increase in weakly dissociated $NaHCO_3$ producing an excess of H+ ions and a concomitant decrease in pH. When the gaseous CO_2 is dissolved, it forms a buffering system with the bicarbonate ions. If a situation exists where the cell density is low or the cells have entered a lag phase, they may not produce enough CO_2 to maintain an optimal pH level. In order to counter such problems, bicarbonate-buffered media require the use of incubators with a 5 - 10% CO_2 atmosphere. Some media with lower bicarbonate levels (e.g., MEM) or with higher levels (DMEM) require ~5% CO_2 and ~10% CO_2, respectively, to maintain a proper pH. The most important factor is the correct percentage of CO_2 based on the particular medium's bicarbonate level, irrespective of cell type.

Cell culture media turns acidic as it becomes exhausted as the acid end products from carbohydrate fermentation lower the pH causing the phenol red to turn from a reddish-purple hue (neutral) to a yellowish hue (acid). The pH status of the medium can be continually monitored as indicated by the color. The breakdown of carbohydrates during fermentation may occur especially by contamination of microorganisms such as bacteria and yeasts yielding incompletely oxidized products. Some forms of fermentation may occur in the absence of oxygen in which case ATP is generated in reaction pathways when organic compounds act as both acceptors and donors of electrons. Factors affecting the pH stability of the cell culture medium include:

- Glucose concentration
- Headspace above the medium to prevent CO_2 loss and an increase in hydroxyl ions
- Buffer capacity & type

In essence, we see that the buffer system is what prevents such a large change in pH. There are many important buffer solutions, and most biochemical reactions, whether they occur in the laboratory or from within the human body, are carried out in buffered solutions. HEPES, in comparison to the sodium bicarbonate buffer system, is more suitable as a buffer when the physiological pH at 7.2 - 7.6 is desired and may be used with or without a CO_2 atmosphere, whereas the bicarbonate buffering system requires

the use of a CO_2 incubator.

Predominant Characteristics

- Zwitterionic buffer
- pKa range between 6.0 and 8.13
- Liquid formulation with high solubility
- Easy-to-use
- For biochemistry, cell-culture, and molecular biology applications

Procedure

- Take a bottle from 15 - 30°C and read the label.
- Wipe the outside of the bottle with a disinfectant solution such as 70% ethanol.
- Pipette appropriate volume using an aseptic/sterile technique under a laminar flow culture hood and work according to established protocols.

Quality Control

HEPES Buffer Solution (1 M) is tested for sterility, pH, osmolality, and endotoxin concentration.

Storage and Stability

The product should be kept at **15 - 30°C**.
The product is **light-sensitive** and therefore should not be left in the light.
Shelf life: 18 months from date of manufacture.

Precaution and Disclaimer

For research use only, not for clinical diagnosis, and treatment.