Buffering Systems

Maintaining the pH of the cell culture medium is critical to cell viability. Most cell lines grow well at pH 7.4 and are inhibited by growth at pH 6.8. Glucose is usually the sugar included in media and is metabolized by the cells very rapidly, at a rate faster than it is needed. Byproducts of this metabolism include pyruvic, lactic acids and CO_2. To reduce the amount of lactic acid glucose can be replaced with other sugars such as galactose or fructose. These sugars are utilized at a slower rate, but also result in a slower rate of cell growth. This replacement can slow down the onset of a pH shift.

The bicarbonate and carbon dioxide buffering system is most commonly used to maintain physiological pH 7.2-7.4 of a culture. Bicarbonate is a weak buffer with a pK_a of 6.1 making a pH range of 7.2-7.6 more difficult to prevent rapid pH changes. Bicarbonate, however, is non-toxic, and has nutritional value.

Bicarbonate/CO_2 Systems

The amount of dissolved CO_2 in water is dependent on the amount of atmospheric CO_2 and the temperature. Increasing CO_2 tension in the absence of sodium bicarbonate leaves the medium acidic.

carbonic acid — bicarbonate

$$[CO_2(g) \leftrightarrow CO_2(aq)] + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-}$$ carbonate

The addition of sodium bicarbonate in the presence of CO_2 will drive the equation above to the left to allow for an equilibrium to be acheived and the pH maintained at 7.2-7.4.

$$NaHCO_3 + H_2O \leftrightarrow Na^+ + HCO_3^- + H_2O \leftrightarrow Na^+ + H_2CO_3 + OH^- \leftrightarrow Na^+ + H_2O + OH^- + [CO_2\,(aq) \leftrightarrow CO_2(g)]$$

Although CO_2 is produced by growing cells, it is not produced in a high enough level when growing at a low cell density or during lag phase to maintain an optimal pH. Use of a CO_2 incubator helps to control the CO_2 tension and the temperature.

Organic Systems

Organic buffers such as HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid) or MOPS (N-morpholino propane sulfonic acid) do not require elevated levels of CO_2 and provide an alternative to the use of bicarbonate for buffering. Unlike the low pK_a of bicarbonate, HEPES has a pK_a of 7.66 and MOPS has a pK_a of 7.2 making them much stronger buffers in the pH 7.2-7.6 range. Disadvantages to using these buffers, however, are toxicity and lack of nutritional value. Recommended usage of HEPES is at concentrations of 10-25 mM; toxicity to cells can occur at a concentration over 100 mM. Usage of MOPS should not exceed 20 mM. They may be used in addition to bicarbonate for added buffering capacity. It is important to maintain sufficient bicarbonate in the medium for nutritional purposes even if organic buffers are being used.

NOTE:

Most cellgro® powdered cell culture media are deficient in sodium bicarbonate. Please refer to our formulations, or to the Sodium Bicarbonate Addition Chart to determine the amount of bicarbonate to supplement to your specific powdered media.

Product Description	Catalog No.	Size:
HEPES, 1M Solution	25-060-CI	6 x 100 mL
HEPES, Powder	61-034-RM	1 x 100 g
	61-034-RO	1 x 500 g
	61-034-RR	1 x 1 kg
Sodium Bicarbonate, 7.5% (w/v) Solution	25-035-CI	6 x 100 mL
Sodium Bicarbonate, Powder	61-065-RO	1 x 500 g
	61-065-RR	1 x 1 kg
MOPS Buffer, Powder	46-103-RM	1 x 100 g

References:

1. Freshney, R. I., ed. *Animal Cell Culture: A Practical Approach*. 2nd ed. New York: Oxford University Press, 1992: 54
2. Davis, J. M., ed. *Basic Cell Culture: A Practical Approach.* New York: Oxford University Press, 1994: 59-60.
3. Freshney, R. Ian. *Culture of Animal Cells: A Manual of Basic Technique.* 3rd ed. New York: Wiley-Liss, 1994: 80-84.