GIBCO™
Invitrogen Corporation

CO_2 Independent Medium

without L-glutamine

CAUTION: **Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB_sAg. Handle in accordance with established bio-safety practices.**

Cat. No.: 18045 **500 mL**

Storage Condition: 2 to 8°C, in the dark.

Background

Traditionally cell culture media have relied on sodium bicarbonate as the primary buffering component.[1] When utilizing a bicarbonate buffer system, a carbon dioxide (CO_2) rich atmosphere of five to ten percent in air is required to maintain the *in vitro* environment at physiological pH.[1,2] However, such a system does not allow for work outside of the CO_2 regulated atmosphere for extended periods of time due to extreme fluctuations in pH, which can affect cellular function.[3,4]

As an alternative to bicarbonate and synthetic buffering systems, GIBCO has developed a CO_2 Independent Medium[5,6] which is capable of maintaining long term pH stability under atmospheric CO_2 (0.04% Figure 1). GIBCO CO_2 Independent Medium contains a unique buffering system composed of mono and dibasic sodium phosphate and ß-glycerophosphate. A small amount of sodium bicarbonate has been included in the formulation to meet essential bicarbonate dependent functions.[7,8,9] No synthetic buffers are utilized, thus eliminating any cytotoxic effects associated with such buffering systems.[10,11] Additionally, GIBCO CO_2 Independent Medium has been formulated with components that enhance cellular production and utilization of CO_2 such that an exogenous source of CO_2 is not required for the maintenance of CO_2 dependent cellular functions.[2,8,9,12]

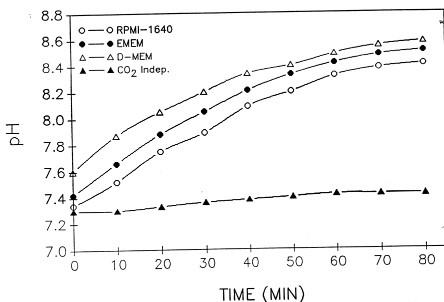

Figure 1. pH stability of CO_2 Independent Medium as compared to other media which utilize a $NaHCO_3$ buffering system. CO_2 Independent Medium has been found to maintain a stable pH over a 72-hour test period in atmospheric CO_2 without the presence of cells.

This product offers the user long term pH stability which allows for the maintenance of normal cellular function during routine culture procedures. Additionally, this medium provides versatility in that it can be utilized in either an open or closed culture system, the latter being ideally suited for use in toxicological and/or virological procedures where there is a risk of aerosol contamination or infection. It is also anticipated that this product would be beneficial for large scale cell culture procedures and for tissue transport.

Quality Control Testing

CO_2 Independent Medium is quantitatively performance tested using a growth assay which monitors the 7-day growth kinetics of WI-38, a human diploid lung fibroblast cell line. Each production lot is further evaluated for pH stability over a 72-hour test period in atmospheric CO_2. Additional standard evaluations are pH, osmolarity, endotoxin and tests for the presence of bacterial and fungal contaminants. See the available Certificate of Analysis for lot-specific test results and product specifications.

Instructions for Use

CO_2 Independent Medium is supplied without L-glutamine and must be supplemented with 4 mM L-glutamine either as powder (584.6 mg/L) or liquid (20 mL of 100X stock, Cat. No. 25030). If a liquid L-glutamine source is used, an equivalent amount of medium should be removed to equal the volumetric amount of L-glutamine added. For the best performance of this product, it is recommended that an equivalent amount of medium also be removed to compensate for the addition of serum. The addition of L-glutamine and serum on top of the original volume may affect product performance.

Applications

For the development of CO_2 Independent Medium a variety of anchorage dependent and suspension cell lines have been utilized. As summarized in Table 1, growth kinetic analysis demonstrated that all of the cell lines tested proliferated at rates equal to or greater than control cultures when grown in a closed, 0% CO_2 culture system. When utilizing an open, 0% CO_2 culture environment, some anchorage dependent cell lines (BHK-21, HT-29 and MDBK) may require adaptation to CO_2 Independent Medium prior to initiation of experiments. If desired, cultures can be placed in a 5% CO_2 incubator utilizing either an open or closed culture system without any deleterious effects to cellular growth.

Table 1: Summary of growth performance analysis of unadapted anchorage dependent and suspension cell lines cultured in CO_2 Independent Medium.

	Culture Environment	
Cell Line	Open System, 0% CO_2	Closed System, 0% CO_2
AE-1	ND	++
BHK-21	+/-	++
CHO	+	++
CHO*	ND	+
HT-29	+/-	+
MDBK**	+/-	++
VERO	+	+
WI-38	+	+

* Adapted to suspension growth in CHO-S-SFM.
** Display normal growth at low seeding density.
\+ Cell growth is equal to the control.
ND Not determined.
++ Cell growth exceeds the control.
+/-Cell growth is slightly lower than control.

Adaptation of Cells to CO_2 Independent Medium

For maximum growth performance, some cell lines may require either direct or sequential adaptation to CO_2 Independent Medium. In either case, the pre-adapted cell line should be in mid-logarithmic growth phase with high (>90%) viability. Success of the adaptation procedure will depend on the cell line being used and the culture conditions employed. It is recommended that the user first evaluate this product with unadapted cells since not all cell lines will require adaptation. If the growth assays employed are conducted in a closed culture system, stock cultures can be directly adapted and maintained in CO_2 Independent Medium using a closed, 0% CO_2 system.

A. Direct Adaptation

For direct adaptation of selected cell lines, stock cultures should be inoculated at normal seeding densities and incubated using a closed cap in a humidified (37°C) incubator with 0% CO_2. Growth should be monitored daily and cells subcultured at 80-90% confluency. Subsequent passages should utilize a humidified (37°C) 0% CO_2 atmosphere with an open cap vessel. If the cell cultures fail to maintain acceptable growth and viability over 3-5 passages during direct adaptation, the sequential adaptation method should be used.

B. Sequential Adaptation

For this procedure cells are initially inoculated into a 50:50 ratio (v/v) of CO_2 Independent Medium and the currently utilized medium. Stock cultures should be maintained under an open cap in a humidified (37°C) incubator with 0% CO_2. Flasks should be subcultured at 80-90% confluency and inoculated with a 75:25 ratio (v/v) of CO_2 Independent Medium and the currently utilized medium. Subsequent subculturing should use 100% CO_2 Independent Medium and maintained as described above.

References:

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3. Mackenzie, C.G., Mackenzie, J.B. and Beck, P. The effect of pH on growth, protein synthesis and lipid-rich particles of cultured mammalian cells. *JBiophys. Biochem. Cyto.* **9**:141-156 (1961).
4. Eagle, H. The effect of environmental pH on the growth of normal and malignantcells. *J. Cell Physiol.* **82**:1-8 (1973).
5. Battista, P.J., DiSorbo, D.M. and Weiss, S.A. Development of a carbon dioxide independent medium. *In Vitro Cell. Dev. Biol.* **27**:120A (1991).
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7. Harris, M. The role of bicarbonate for outgrowth of chick heart fibroblasts invitro. *J. Exper. Zool.* **125**:85-98. (1954).
8. Geyer, R.P. and Chang, R.S. Bicarbonate as an essential nutrient for human cells in vitro. *Arch. Biochem. Biophys.* **73**:500-506. (1958).
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10. Spierenburg, G.T., Oerlemans, F.J., Van Laarhoven, J.M. and deBruyn, C.M.Phototoxicity of N-2 hydroxyethyl-piperazine-N-2 ethanesulfonic acid-buffered culture media for human leukemia cell lines. *Cancer res.***44**:2253-2254 (1984).
11. Zigler, J.S., Lepe Zuniga, J.L., Vistica, B. and Gery, I. Analysis of the cytotoxic effects of light-exposed HEPES-containing culture medium. *InVitro Cell. Dev. Biol.* **21**:282-287 (1985).
12. Chang, R.S., Liepins, H. and Margolish, M. Carbon dioxide requirement and nucleic acid metabolism of HeLa and conjunctival cells. *Proc. Soc. Exper. BiolzMed.* **106**:149-152 (1961).

For further information on this or other GIBCO™ products, contact Technical Services at the following:

United States TECH-LINE℠ : 1 800 955 6288
Canada TECH-LINE: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

For research use only.
CAUTION: Not intended for human or animal diagnostic or therapeutic uses

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