gibco
by life technologies

CO_2 Independent Medium

Description

CO_2 Independent Medium is a non-HEPES proprietary medium used for supporting cell growth for a variety of suspension and adherent mammalian cells such as epithelial, fibroblast, and lymphoid cell lines without a CO_2 incubator. CO_2 Independent Medium is ideally suited for transporting cells or tissue, handling of mouse embryos under atmospheric conditions, and for use in toxicological and/or virological procedures where there is a risk of aerosol contamination or infection. CO_2 Independent Medium contains a unique buffering system composed of mono and dibasic sodium phosphate and β-glycerophosphate capable of maintaining long term pH stability under atmospheric CO_2 (0.04%). A small amount of sodium bicarbonate has been included in the formulation to meet essential bicarbonate dependent functions. No synthetic buffers are utilized, thus eliminating any cytotoxic effects associated with such buffering systems. Additionally, 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.

Product	Catalog no.	Amount	Storage	Shelf life*
CO_2 Independent Medium	18045-088 18045-054** 18045-070**	500 mL 500 mL 10 × 500 mL	2°C to 8°C; Protect from light	12 months

* Shelf Life duration is determined from Date of Manufacture.
** Note: For European customers only.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important information

- CO_2 Independent Medium requires supplementation with GlutaMAX™-I or L-glutamine and when necessary, fetal bovine serum (FBS).
- 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.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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.

Prepare medium

1. Aseptically add 20 mL GlutaMAX™-I (200 mM) or L-glutamine (200 mM) to 880 mL CO_2 Independent Medium before use.
2. Aseptically add 100 mL FBS to the medium before use.
3. Add antibiotics, if required.

Culture conditions

Media: complete CO_2 Independent Medium

Cells: mammalian

Culture type: Adherent or Suspension

Culture vessels: T-Flasks

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 0% CO_2 in air. Open or closed culture systems minimizing exposure of cultures to light.

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.

Direct adaptation

1. Inoculate cultures at normal seeding densities and incubate using a closed cap in a humidified (37°C) incubator with 0% CO_2.
2. Monitor cell growth daily and subculture cells when they reach 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, use the sequential adaptation method.

Sequential adaptation

Note: Multiple passages at each step may be required.

1. Inoculate cells at normal seeding densities into a 50:50 ratio (v/v) of CO_2 Independent Medium and the currently utilized medium.
2. Maintain cultures under an open cap in a humidified (37°C) incubator with 0% CO_2.
3. Monitor cell growth daily and subculture cells when they reach 80–90% confluency into a 75:25 ratio (v/v) of CO_2 Independent Medium and the currently utilized medium.
4. Monitor cell growth daily and subculture cells when they reach 80–90% confluency into 100% CO_2 Independent Medium.

Subsequent subculturing should use 100% CO_2 Independent Medium and maintained as described in the preceding adaptation procedure.

Related products

Product	Catalog no.
L-Glutamine, 200 mM (100X), liquid	25030
GlutaMAX™-I, 200 mM (100X), liquid	35050
Certified FBS, Heat Inactivated, US	10082
Antibiotic-Antimycotic (100X), liquid	15240
Fungizone® Antimycotic, liquid	15290
Gentamicin	15750
Penicillin-Streptomycin, liquid	15140
Dulbecco's Phosphate Buffered Saline, without calcium and magnesium	14190
TrypLE™ Express (1X), liquid, without Phenol Red	12563
Trypsin-EDTA, 1X	25300
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of symbols and warnings

The symbols present on the product label are explained below:

				
Temperature Limitation	Manufacturer	Batch code	Use By:	Catalog number
				
Caution, consult accompanying documents	Consult instructions for use	Keep away from light	Sterilized using aseptic processing techniques	

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

References

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).

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**