

OPTI-MEM® I Reduced Serum Medium modification of MEM (Eagle's)

Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV and HB $_{\rm S}$ Ag. Handle in accordance with CAUTION:

established bio-safety practices.

100 mL Cat. No.: 31985 500 mL

Component-Deficient Media: Cat. No.: *11058

500 mL without phenol red

> with GLUTAMAX™ I 51985 500 mL

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

Shelf Life: See product label for expiration date.

NOTE: For catalog number 11058 please also refer to Component-Deficient

Medium Supplementation below.

OPTI-MEM I is a versatile, chemically-defined medium, formulated to reduce significantly the amount of serum required for cultivating mammalian cells in vitro.

OPTI-MEM I is a multi-purpose medium that has proven useful in reducing serum requirements for a wide variety of cell lines and applications. It has been shown effective in the growth and maintenance of both adherent and non-adherent cell lines. When supplemented with 2-4% fetal bovine serum or alternative sera, OPTI-MEM I supports proliferative rates and maximal cell densities comparable to, and in some cases superior to, conventional media supplemented with 10% fetal bovine serum. Relatively non-fastidious cell lines may be maintained in long-term culture with even more substantial serum reduction. If using adherent cell lines and less than 2% serum supplementation or in an agitated system such as in roller bottles, the medium should be further supplemented with 100 mg/L CaCl₂.

The versatility of OPTI-MEM I in the propagation of various cell types makes this medium the optimal choice for many cell culture requirements.

The raw materials used to produce OPTI-MEM I are screened by ${\sf GiBCO}^{\sf TM}$ rigorous quality standards. The complete medium is performance tested to ensure the lot-to-lot consistency required for research and production applications.

The shelf life of OPTI-MEM I is comparable to conventional glutamine-containing formulations. This stability permits laboratories to stock and maintain inventories of OPTI-MEM I compatible with routine laboratory practices.

OPTI-MEM I is a modification of Eagle's Minimal Essential Medium, buffered with HEPES and sodium bicarbonate, and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine or GLUTAMAX, trace elements and growth factors. The protein level is minimal (15 µg/mL), with insulin and transferrin being the only protein supplements. Phenol red is included at a reduced concentration as a pH indicator. OPTI-MEM I may be supplemented with 2-mercaptoethanol prior to use. CaCl₂ level is reduced to 99.9 mg/L for convenience in working with hybridomas.

Instructions for Use

Conversion to OPTI-MEM I

Converting to OPTI-MEM I is easy. For most applications, no weaning procedures are necessary to attain 50% reduction in serum supplementation when converting to OPTI-MEM I. The conversion can be made by simply centrifuging the cells, decanting the supernatant, and resuspending them in OPTI-MEM I with the reduced serum supplementation. (See the tables in the Applications section for typical serum supplementation reduction). Additional serum reduction may be realized with minimal weaning. The optimal serum supplementation for each specific application should be determined based on the performance characteristics expected (growth promotion, secondary metabolite production, etc.). Extended use of OPTI-MEM I in the maintenance of cell lines has shown no loss of viability or growth rate. Keep OPTI-MEM I away from direct light.

Use of 2-Mercaptoethanol

2-mercaptoethanol is a reducing agent which has been shown to enhance growth ¹, plating efficiency ² and/or antibody synthesis ³ of specific cell lines. The kinetics of this phenomenon are not fully understood. Most applications do not require supplementation with 2-mercaptoethanol.

Since OPTI-MEM I is functional in a broad range of applications with numerous cell types, it is prudent to evaluate the utility of 2-mercaptoethanol supplementation based on the specific application.

2-mercaptoethanol is available separately as a 1000X concentrate (5.5x10 -2M) in Dulbecco's Phosphate Buffered Saline without calcium and magnesium 21985, 50 mL. It may be added to OPTI-MEM I immediately prior to use. OPTI-MEM I is stable for two months following 2mercaptoethanol addition, provided that this period does not exceed the expiration date stated on the OPTI-MEM I or 2-mercaptoethanol labels

If desired, aseptically add 0.5 mL 2-mercaptoethanol (1000X) to each 500 mL of OPTI-MEM I. Do not pipette by mouth. Mix thoroughly by swirling (do not shake). Store unused portions of 2-mercaptoethanol tightly capped in the original container. The final concentration of 2-mercaptoethanol in OPTI-MEM I will be $5.5x10^{-5}\,\mathrm{M}$.

Most cells routinely cultured in serum-supplemented medium may be directly transferred into OPTI-MEM I with a minimum of 50% reduction in serum.

Substantial further reduction in serum requirement has been achieved with various murine myeloma (SP2/0-Ag14, P3X63-Ag8.653, P3-NS1-Ag4-1) and derived hybridomas.

We have also achieved significant reduction in the amount of serum required for growing fibroblasts

and epithelial cells of normal and tumor origin. If using adherent cell lines and less than 2% serum supplementation or in an agitated system such as in roller bottles, the medium should be further supplemented with 100 mg/L CaCl₂.

GIBCO research has established that supplementation with fetal bovine serum (FBS) may be reduced when using OPTI-MEM I, to the levels indicated below for the following cell lines, while maintaining growth rates comparable to basal media at higher serum supplementation levels.

Cell Types	% FBS in OPTI-MEM I
Hybridoma Technology - Mouse and Human	
Fusion	4
Cloning	2 - 4
Growth and Ab production - Myelomas and Established Hybridomas	0.5 - 2
Diploid Fibroblast Cell Lines	2 - 4
Primary Fibroblasts	2 - 4
Rat and Hamster Embryo Cell Lines	2
Lymphoblastoid Cell Lines	0.5 - 2
Monkey Kidney Cells	4
Human and Bovine Embryonic Kidney Cells	2 - 4

Supplementation of OPTI-MEM I with alternative mammalian sera has also demonstrated impressive results. OPTI-MEM I supplemented with 4% alternative sera has performed comparable to, in some cases superior to, basal media supplemented with 10% FBS in the following applications:

Application	Cell Line	Serum Alternative at 4%
Growth Promotion	Sp2/0-Ag14 (Sp2)	Calf, Horse
	AE-1 (Sp2 derived Hybridoma)	Calf, Horse
	CHO	Horse
	BHK-21	Calf, Horse
Cloning	Sp2	Calf, Newborn Calf, Horse
	P3x63-Ag8.653 (653)	Calf, Newborn Calf, Horse
		Calf, Horse
Plating	653	Calf, Horse
	BHK-21	Calf, Horse
	CHO	Calf, Newborn Calf
MAb Production	AE-1	Calf, Newborn Calf, Horse

In limited studies, electroporation in OPTI-MEM I has yielded higher levels of transient gene expression and cell viability than typical electroporation medium. OPTI-MEM I, with 2-mercaptoethanol has been reported to improve transfection efficiency in CV-1 and COS 1 cells transfected with a monkey SV-40-like promotion vector. 4.5

It is our intent to update periodically, application of OPTI-MEM I for specific cellular requirements. We would appreciate if researchers using OPTI-MEM I share their experiences with GIBCO, so that the information gained can be shared with the scientific community.

Component-Deficient Medium Supplementation

When using a component-deficient variation of OPTI-MEM I, the following supplementation concentration will be consistent with the original formulation:

Cat. No. 11058 phenol red at 0.00160 gm/L

Quality Control

OPTI-MEM I is subjected to pH, osmolality, endotoxin, bacterial, fungal, and mycoplasma testing. The endotoxin level is less than 1.0 EU/mL.

Each lot of OPTI-MEM I is evaluated utilizing sensitive quantitative assays for its ability to support cloning efficiency of a murine myeloma cell line, and growth over multiple subcultures of an adherent cell line. Test lots of OPTI-MEM I at 2% (CHO growth) and 4% (Sp2 cloning) serum supplementation are compared to a previously approved OPTI-MEM I control.

GIBCO cell culture liquid products are prepared by an aseptic process for which each step has been validated to ensure that all products meet the industry standard sterility assurance level of 10^3 ; i.e., product that demonstrates a contamination level of no more than 1 of 1000 units during the manufacturing process. The highest level of sterility assurance (equal to or greater than 10^6) cannot be achieved without terminal sterilization which is harmful to the performance of cell culture products.

Storage

As proper packaging for chemically defined media is of utmost importance, we recommend the use of the original containers for full-term storage.

For optimal performance, OPTI-MEM I should be stored 2 to 8°C in the dark prior to use

OPTI-MEM I is also available in economical powdered form. Refer to the GIBCO catalog or contact your Invitrogen sales representative for details.

Cells, Alan

Jayme, David W. and Blackman, Kenneth E., Cell Culture Media for Propagation of Mammalian Viruses and Other Biologicals. Advances in Biotechnological Processes. vol 5, pp. 1-30. R. Liss, Inc. New York (1985).

R. Liss, Inc. New York (1985).

²Kawamoto, Sato, J.D., Le, A. McClure, D., Sato, G. Development of a Serum- Free Medium for Growth of NS-1 Mouse Myeloma Cells and Its Application to the Isolation of NS-1 Hybridomas.

³Mishell, B.B. and Mishell R.I., Primary Immunization in Suspension Cultures. Selected Methods in Cellular Immunology. Freeman (1980).

⁴Saffer, J.D. and Hughes, D.L. Nuclear Acids Research. 14, 3604 (1986).

⁵Livelli, T. personal communication (1987).

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

United States TECH-LINE SM: 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 in vitro diagnostic use. CAUTION: Not for human or animal therapeutic use Uses other than the labeled intended use may be a violation of local law.

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

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