GIBCO™
Invitrogen Corporation

OPTI-MEM®I
Reduced Serum Medium
Modification of MEM (Eagle's), powder

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.: **22600** **10 X 1 L**
10 L

Storage Condition: 2 to 8°C, dark and dry.
Shelf Life: See product label for expiration date.

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.

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 Invitrogen's 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.

Formulation

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, 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.

Preparation of 1X Medium

1. Measure out 5% less distilled water than required total volume of medium being prepared. Use a mixing container as close to the final volume as possible. **NOTE: Very high quality distilled water is required to achieve optimal cell growth in reduced serum systems.**
2. Add powdered medium to 20 to 30°C (room temperature) water with gentle stirring. Do not heat water.
3. Rinse out inside of package to remove all traces of powder.
4. Allow to stir until medium dissolves completely. (Usually achieved within five minutes).
5. Add 2.4 grams Sodium Bicarbonate ($NaHCO_3$), reagent grade per liter of medium.
6. Dilute to required volume with water. Stir gently until all components are dissolved and medium is thoroughly mixed. (Do not over mix).
7. pH Adjustment
   a. Adjust pH of medium 0.2-0.3 units below desired final pH. The recommended final pH of OPTI-MEM I after filtration is 7.3 ± 0.1. pH will generally rise 0.1 - 0.3 units upon filtration.
   b. Use of 1N NaOH or 1N HCl is recommended for pH adjustment. Add slowly with stirring and constant pH monitoring.
   c. After pH has been adjusted, keep container closed until medium is filtered.
8. Process immediately by membrane filtration. Positive pressure and 0.2 µ membrane porosity are recommended.

NOTE: Use low protein absorbent filter. Flush filter with sufficient media to remove filter residue prior to dispensing into final container.

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.

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 ($5.5x10^{-2}$ M) in Dulbecco's Phosphate Buffered Saline without calcium and magnesium (Cat. No. 21985). It may be added to OPTI-MEM I during medium preparation (after procedural step No. 5) or immediately prior to use. If desired, add 1 mL 2-mercaptoethanol (1000X) per liter of OPTI-MEM I. Do not pipette by mouth. Mix thoroughly (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 $5.5x10^{-5}$M. OPTI-MEM I is stable for two months following 2-mercaptoethanol addition, provided that this period does not exceed the expiration dates stated on the OPTI-MEM I or 2-mercaptoethanol labels.

Applications Information

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.

Invitrogen 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
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, applications of OPTI-MEM I for specific cellular requirements. We would appreciate if researchers using OPTI-MEM I, share their experiences with Invitrogen, so that the information gained can be shared with the scientific community.

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.

Storage and Shelf Life

Store reconstituted OPTI -MEM I at 2 to 8°C in the dark. To achieve optimal results, 1X liquid OPTI-MEM I medium should be used within 4-6 months provided that date does not exceed the expiration date of the powdered medium used.
OPTI-MEM I is also available in convenient liquid form.

References:

1. Jayme, David W. and Blackman, Kenneth E., Cell Culture Media for Propagation of Mammalian Cells, Viruses and Other Biologicals. *Advances in Biotechnological Processes.* vol 5, pp. 1-30. Alan R. Liss, Inc. New York(1985).
2. Kawamoto, 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. *Analytical Biochemistry.* **130,** 445-453 (1983).
3. Mishell, B.B. and Mishell R.I., Primary Immunization in Suspension Cultures. *Selected Methods in Cellular Immunology.* Freeman (1980).
4. Saffer, J.D. and Hughes, D.L. *Nuclear Acids Research.* **14,** 3604 (1986).
5. Livelli, T. personal communication (1987).

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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.

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