

## HL-1™ SUPPLEMENT

### Instructions for use

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#### Description

HL-1™ Supplement is a chemically defined medium additive that can be used to replace serum or significantly reduce its concentration in a variety of basal media. It contains less than 30mcg protein/ml when diluted 1:100 in medium and it does not contain bovine serum albumin or other undefined protein ingredients. HL-1™ Supplement, when added to most basal media, will support the growth of mouse hybridoma cells and certain other differentiated cells of lymphoid origin (see Applications section).

#### Storage and Stability

HL-1™ Supplement can be stored at either room temperature or 4°C. Under either condition, the shelf life of HL-1™ Supplement is at least 300 days from the date of manufacture. Once diluted into basal medium, it should be used within 45 days.

NOTE: If the preparation is stored at 4°C, a slight turbidity may occur. This will disappear after solution is brought to room temperature. Do not pre-warm in waterbath or incubator. Once opened, unused portions of HL-1™ Supplement can be stored at 4°C or room temperature. It is recommended that the product not be frozen as the growth-promoting properties may be inactivated.

#### Instructions for Use

1. Aseptically add 10 ml of HL-1™ Supplement to 1 liter of the sterile basal medium of choice. The recommended basal media are:
  1. 1:1 mixture of Dulbecco's Modified Eagle Medium (DMEM) and Ham's F12 (high glucose)
  2. RPMI-1640
  3. Iscove's Modified Dulbecco's Medium (IMDM)
  4. DMEM containing up to 1% fetal bovine serum (FBS)
  5. Other enriched media containing amino acids.

The medium should also be supplemented with L-glutamine (2-4mM), sodium Pyruvate (1-2mM), and sodium bicarbonate (2.2 g/L or 1.2 g/L), if not already present. The pH of the basal medium should be that recommended by the manufacturer (typically pH 7.2), as the pH will not change after the addition of the HL-1™ Supplement. Since HL-1™ Supplement is sterile, filtration of sterile medium following its addition is not necessary.

2. Antibiotics and pH stabilizing buffers such as HEPES or MOPS may be added at the user's discretion. It should be noted, however, that these additives may affect cell growth since some cells appear to become more sensitive to these agents under serum-free conditions.

#### Adaptation of Serum-Dependent Cells

Weaning cell lines from serum-containing media may be recommended. The following protocol will aid adaptation to a serum-free environment in media containing HL-1™ Supplement by gradual reduction of the serum concentration:

1. Week 1 – Reduce the serum concentration to 5% FBS.
2. Week 2 – Reduce the serum concentration to 2% FBS and add HL-1™ Supplement at 1%.
3. Week 3 – Reduce the serum concentration to 1% FBS with HL-1™ Supplement at 1%. See note below.
4. Week 4 – Reduce the serum concentration to 0.5% FBS with HL-1™ Supplement at 1%. See note below.
5. Week 5 – Reduce the serum concentration to 0.25% FBS with HL-1™ Supplement at 1%. See note below.
6. Week 6 – Eliminate FBS and culture cells in serum-free HL-1™ Supplement-containing medium.

NOTE: At each reduction step, cells will evidence an initial lag in growth rate. Passage the cells three times per week during the adaptation period,

seeding at a density of  $1-2 \times 10^5$  cells/ml; do not allow densities to exceed  $8-10 \times 10^5$  cells/ml. Upon reduction to the 0.5% serum concentration, a greater lag in the growth rate may be observed. Under these conditions, a higher seeding density and less frequent passaging may be required until cells resume their normal growth characteristics.

## Adaptation of Serum-Dependent Cells

When measuring sample media supernatants from hybridoma cultures for monoclonal antibody secretion levels using ELISA, Tween® 20 should be added to the sample aliquots and the wash fluid. The final concentration of Tween® 20 should be 0.05% in these solutions.

## HL-1™ medium has successfully been tested on the following cell lines:

### Transformed & Established Cell Lines

BB88	murine	erythroid (leukemia)
U937	human	macrophage
P815	murine	macrophage
P388D1	murine	macrophage
WeHi3*	murine	monocyte
JLS-V5	murine	spleen cell
RaJi*	human	B lymphoblastic
GCL2	hamster/ mouse	B lymphoma X Normal B
70Z-3	murine	Pre-B lymphoma
70Z/3.12	murine	B lymphoma
S49 and variants	murine	T lymphoma
RAW309F1.1	murine	T lymphoma
WeHi7	murine	T lymphoma
L5178Y	murine (DBA/2)	Lymphoma
I-10	murine	Leydig-tumor
MCF-7 (NIH)	human	breast carcinoma
MCF-7 (MCF)	human	breast carcinoma
NIH ZR-75	human	breast carcinoma
COLO 302 HSR	human	colon carcinoma
J82	human	bladder carcinoma
SW 1738	human	bladder carcinoma
SW780	human	bladder carcinoma
EL4	murine	T lymphoma
RL1	murine	T lymphoma
BW5147.3	murine	T lymphoma
LBRM-33	murine	T lymphoma
Friend leukemia	murine	leukemia
CCL 119	human	lymphoid
CCL 213	human	Burkitt lymphoma
C91/PL	human	T lymphoma
undesignated	human	astrocytoma

undesignated	human	hepatoma
VERO*	African green monkey	fibroblast
MDCK*	canine	Madin Darby canine kidney
MOLT-3	human	acute lymphoblastic leukemia
MOLT-4	human	acute lymphoblastic leukemia
NAMALWA	human	Burkitt lymphoma
C57BL6	murine (C57 X DBA)	Embryo
CHO K1*	Chinese hamster	Ovary (epithelial-like)

### Hybridomas

HB44*	murine	Sp2/0-Ag14.
HB45*	murine	Sp2/0-Ag14.
HB56*	murine	NS-1
HB59*	murine	NS-1
HB60*	murine	P3X63Ag 8.653
53-7.313	murine	NS-1
MI/9.3.4HL-2	murine	NS-1
8A1	human	CLLC
MI/70.15.1	murine	NS-1
ARB	murine	hybridoma
TIB 175	rat/mouse	S194
TIB 104	rat/mouse	NS-1
TIB 105	rat/mouse	NS-1
TIB 109*	rat/mouse	NS-1
TIB 128	rat/mouse	NS-1
TIB 166	rat/mouse	NS-1
TIB 168	rat/mouse	NS-1
BCS12	murine	P3X63Ag 8.653
BCS 2002*	murine	P3X63Ag 8.653
undesignated	human	WI-L2-729-HF2
undesignated	human	LICR-LON-HMY2
RS	rat/mouse	P3X63Ag 8.653
P3U	murine	P3X63Ag 8.653
undesignated	murine	NS-1
undesignated	murine	P3X63Ag 8.653

### Primary Cells

Human peripheral blood mononuclear  
Mink lymphocytes  
Human fetal adrenal  
Human blood monocytes  
Human peripheral blood T lymphocytes

### Protozoans

*Crithidea fasciculata*  
*Trichomonas augusta*

### Green algae

*Cosmarium botrytis*

\*Cell growth evaluations were performed at Ventrex Laboratories. Information for all other cell lines was provided to Ventrex from outside sources. This product has since been acquired by Lonza, Inc.

## Product Use Statement

**THESE PRODUCTS ARE FOR RESEARCH USE ONLY.** Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

## Ordering Information

HL-1™ Supplement (100X)  
77227                    10 ml

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