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BioWhittaker™ HL-1™ Chemically Defined Serum-free Media

Instructions for Use

Description

HL-1™ chemically defined, serum-free media is a culture medium containing less than 30 μg protein per ml. Components of HL-1™ medium include Water for Injection, HEPES Buffer, known amounts of insulin, transferrin, testosterone, sodium selenite, ethanolamine, a variety of saturated and unsaturated fatty acids and proprietary stabilizing proteins. It contains no bovine serum albumin or other undefined protein mixtures. HL-1™ Medium will support the serum-free growth of various hybridomas and certain other differentiated cells of lymphoid origin.

HL-1™ Medium is available in a powder format. An HL-1™ Chemically Defined, Serum-free Supplement is also available. The HL-1™ Supplement is a medium additive that can be used to replace serum or significantly reduce its concentration in a variety of basal media.

Instructions for Use

1. Immediately prior to use, add sterile L-glutamine (final concentration: 2-4 mM) to HL-1™ Medium.
2. Since cells grown in serum-free media appear to become more sensitive to drugs or antibiotics, one should practice caution when using such additives together with HL-1™ Medium.
3. Prior to introducing cells to HL-1™ Medium, culture vessels containing HL-1™ Medium should be equilibrated in a CO_2 atmosphere (5%-7%) at 37℃. This is a precautionary measure to assure that cells, upon introduction to HL-1™, do not experience temperature and/or pH shock. Pre-warming HL-1™ Medium in a 37℃ water bath is **not** recommended.
4. Remove cells from their current culture medium by gentle centrifugation (200 x g, 5 minutes).
5. Resuspend cells in HL-1™ Medium (supplemented and pre-equilibrated as described above, steps 1-3) at a final cell density of 6-8 $x10^4$ cells/ml. Cells previously carried in serum-containing media may require an adaptation period during which the final concentration of serum is reduced to zero. The time required for this process varies with the cell type (see adaptation section).
6. Incubate the cultures at 37℃ in a humidified atmosphere containing 5%-7% CO_2 in air.
7. Subculture cells growing in HL-1™ Medium when densities approach 8 $x10^5$ to 1 $x10^6$ cells/ml: subculture by diluting cells directly with freshly prepared HL-1™ Medium (see steps 1-3 above).

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 HL-1™ Medium by gradual reduction of serum concentration:

1. Week 1: Reduce the serum concentration to 5% FBS in HL-1™ Medium.
2. Week 2: Reduce the serum concentration to 2% FBS in HL-1™ Medium.
3. Week 3: Reduce the serum concentration to 1% FBS in HL-1™ Medium.
4. Week 4: Reduce the serum concentration to 0.5% FBS in HL-1™ Medium. See note below.

5. Week 5: Reduce the serum concentration to 0.25% FBS in HL-1™ Medium. See note below.
6. Week 6: Eliminate FBS in HL-1™ Medium and culture cells in serum-free HL-1™ Medium.

NOTE: At each reduction stage, cells may show evidence of an initial lag in growth rate. Pass the cells three times per week during the adaptation period, seeding at a density of 1-2 x 10^5 cells/ml. Do not allow densities to exceed 8-10 x 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.

Applications

HL-1™ Medium supports the serum-free growth of many hybridomas, including those derived from the myelomas P3X63Ag8.653 and Sp2/0-Ag14. Other cell types observed to grow in HL-1™ Medium include MDCK, T lymphoma cell lines, certain T-cell hybridomas, etc. For the long-term maintenance of the myeloma NS-1 and certain NS-1 derived hybridomas cultures, a supplement of 0.5% fetal calf serum may be recommended. Myelomas such as P3X63Ag8.653 and Sp2/0-Ag14 can be grown in HL-1™ Medium containing 0.5% and 1.0% fetal calf serum, respectively.

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

HL-1™ Medium Has Been Successfully 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

Transformed & Established Cell Lines

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*	hamster	Chinese hamster ovary (epith.-like)
THP-1	human	monocytic leukemia

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
P3U	murine	P3X63Ag 8.653
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
RS	rat/mouse	P3X63Ag 8.653
BCS12	murine	P3X63Ag 8.653
BCS 2002*	murine	P3X63Ag 8.653
undesignated	human	WI-L2-729-HF2
undesignated	human	LICR-LON-HMY2
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

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

Storage and Stability

HL-1™ Medium is stable for 365 days from the date of manufacture. Freezing HL-1™ Medium is not recommended as this may inactivate its growth-promoting properties. HL-1™ Medium should be stored at 2℃ to 8℃.

Ordering Information

Cat. No.	Description	Size
77201	HL-1™ Medium; chemically defined, serum-free medium	2 x 500 ml
77204	HL-1™ Medium; chemically defined, serum-free medium, powder	50 L
77227	HL-1™ Supplement; chemically defined, serum-free medium supplement (100X)	10 ml

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.

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