Product Profile

Product Name:	*HAT Supplement, Conc.,(50X)*
Catalog Number	*03-080-1-*
Unit Size Availability:	(B) 100ml
Formulation:	*Frozen Liquid*
Defined Storage Conditions:	-20°C
Stability: (Under Defined Handling & Storage Conditions)	Please refer To Product Label

Important Note! Please read the *MSDS* and *Product Profile* carefully in their entirety *before* using this material for possible safety precautions and potential hazards.

Product Description:

HAT Supplement(50X)is a selective growth medium for Mammalian cell culture utilized for the selection of hybrid somatic cell lines, as in the production of monoclonal antibodies. It contains hypoxanthine, aminopterin,the folate antagonist and thymidine. HAT relies on the combination of aminopterin that acts as a folate metabolism inhibitor of dihydrofolate reductase, with hypoxanthine and thymidine(i.e. intermediates of DNA Synthesis); one a purine derivative and the other a deoxynucleoside respectively. Only cell lines expressing both hypoxanthine phosphoribosyl transferase (HPRT+) and thymidine kinase(TK+) can survive in this medium. Aminopterin inhibits *de novo* synthesis of nucleosides, while HPRT and TK supply them from hypoxanthine and thymidine. DNA synthesis requires synthesis of four (4) nucleotides (ATP, CTP, GTP and TTP). Aminopterin blocks the synthesis of GTP and TTP resulting in the absence of DNA synthesis. Thymidine allows for TTP production. Although on the one hand, B-Lymphocytes can use hypoxanthine to make GTP due to the enzyme, HGPRT, nevertheless, they will expire in tissue culture within 7-10 days. Myeloma cells, on the other, cannot use hypoxanthine (HGPRT enzyme-deficient) but will also expire in HAT Medium due to their inability to make GTP.

Hybridoma cells can use hypoxanthine to make GTP and therefore can survive in cell culture. HGPRT is an enzyme in purine metabolism which functions to salvage purines from degraded DNA for renewed purine synthesis. In this function, it acts as a catalyst in the reaction between Guanine and Phosphoribosyl pyrophosphate (PRPP) to form GMP. B-Cells contain HGPRT which enables them to survive when fused to Myeloma cells when grown on HAT medium to produce Monoclonal Antibodies.

Monoclonal Antibodies

This process for the preparation of Monoclonal Antibodies is known as Hybridoma technology. The antibodies are produced from cells called Hybridoma cells. A Hybridoma is often considered a hybrid cell and is produced by the injection of a specific antigen into a mouse that is first exposed to an antigen to which particular antibody isolation is of interest. Once the splenocytes are procured from the antibody-producing cell and isolated, and the subsequent fusion with a cancerous myeloma cell resulting in a hybrid cell which then can be cloned to further create many identical daughter clones. It is then that these daughter clones then secrete the immune cell product. The B-cells are fused with HGRPT negative, immortalized myeloma cells using Polyethylene Glycol (PEG) or a Sendai virus. The fused cells are then incubated in the HAT medium. Hence, unfused myeloma cells die as they cannot produce nucleotides because of the aminopterin in the medium. The unfused B-cells die as they have a short life-span and in this way, only the B-cell myeloma hybrids survive. These cells produce now produce antibodies,a characteristic of B-cells and are immortalized which is a characteristic of myeloma cells. Since the incubated medium is diluted into multi-wall plates and the antibodies are produced by the same B-cell, they will be directed towards the same antigenic determinant (epitope) and are thus known as monoclonal antibodies. The appearance of hybrid clones varies within 2-3 weeks after fusion. It is of the utmost importance that a newly established hybridoma is cloned thoroughly to ensure that the cells growing in culture are of monoclonal origin and not a mixture of two or more hybridomas. Such a hybridoma mixture will likely result in a gradual decline of specific antibody production due to the overgrowth of contaminating hybridomas. Since the supernatant in each well can be checked for desired antibody, the cultured supernatants from the hybridomas should be tested for specific antibody production.

The ruse here is that aminopterin blocks DNA *de novo* synthesis that is absolutely essential in order for cell division to proceed. The other components provide cells with the raw materials to evade the blockage by way of the so-called "salvage pathway" provided that the precise enzymes are present which means having functioning copies of the genes that encode them. HAT medium, the cells are forced to use these exogenous bases, via the salvage pathways as their sole source of purines and pyrimidines. Parental cells either deficient in the enzymes, HGPRT (Hypoxanthine-Guanine Phosphoribosyl Transferase) or Thymidine Kinase(TK) can be eliminated concomitantly as the hybrids grow. After dilution, HAT is suitable for use as a post-infusion selective medium to eliminate unfused or self-fused HGPRT Myeloma Cells. In sum, it can be said that the use of HAT medium for cell culture applications is a form of artificial selection for cells containing working TK and HGPRT.

HGPRT & TK

HGPRT is an enzyme which plays a very important role in the making ATP and GTP from Guanine—the only way guanine, adenine or other purine molecules are able to become part of nucleic acids. Biologists study gene action via the incorporation of modified nitrogenous bases into DNA by manipulating the metabolic roles which this enzyme plays. Similarly, the enzyme TK (Thymidine Kinase) is used for the same purpose due to the fact that it fulfills the same important and unique function for the pyrimidines(e.g. cytosine,thymine,uracil *stc*.).

Predominant Characteristics of HAT Supplement (*50X) include:*

- § Endotoxin and Cell-Culture-Tested
- § Suitable for Cell-Culture Applications
- § Long-Storage When Handled Properly Under Defined Conditions

Storage & Stability:

This product should be stored under specified conditions and used within the timeframe as specified on the product label. D*o not* use after the expiration date as specified on the label. Deterioration of liquid media may be recognized by any of the following characteristics, among others including: (a). color change, (b). granulation/ clumping, (c). insolubility, (d). and/or decrease in expected performance parameters. Any material described above should not be used and therefore discarded. Aminopterin is light-sensitive, store as aliquots, frozen and in the dark..

Quality Control*(Please Note That Each Batch/Lot Will Differ as to the Final Specifications)

Test	Specifications*
Cell Culture Test::	Test & Record
Endotoxins:	Test & Record
Osmolality:	275-290 mOsm/kg
pH:	9.0-10.0
Sterility:	Sterile

Auxiliary Products

Product Name	Catalog Number	Storage Temperature
Dulbecco's Phosphate Buffered Saline(DPBS) without Calcium and Magnesium	02-023-1	Room Temperature(15-30°)
L-Glutamine Solution 29.2mg/ml in Saline	03-020-1	-20°C
L-Alanyl-L-Glutamine Solution(A Dipeptide Substitute)	03-022-1	-20°C
Penicillin-Streptomycin Solution,10,000 units/ml Penicillin G Sodium Salt,10mg/ml Streptomycin	03-031-1	-20°C
HT Supplement, Conc.,50X	03-085-1	-20°C
Fetal Bovine Serum	04-001-1	-20°C
Fetal Bovine Serum(Qualified for Human Embryonic Stem Cells)	04-002-1	-20°C
Adult Bovine Serum	04-003-1	-20°C
Sterile Culture-Grade Water	03-055-1	Room Temperature(15-30°)
Cell Dissociation Solution, Non-Enzymatic	03-071-1	2-8°C
Serum-Free Cell Freezing Medium	05-065-1	2-8°C
Note: For a list of other Antibiotics, Serum, Reagents and Supplements, please refer to our Product Catalog, Product Profiles, Product Guides and Internet Site.		

References:

1) Current Edition Merck Index
2) Biological Industries (BI)Specifications
3) Current Edition USP/E Ph
4) Martindale The Extra Pharmacopeia,28th Edition, Royal Pharmaceutical Society: London, England
5) Darling, D. C. and Morgan, S. J. Animal Cells: Culture and Media ,John Wiley & Sons: New York,1994
6) Hurrell,John G. R. Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press: Boca Raton, Florida