Product Profile

Product Name:	*HT Supplement, Conc.,(50X)*
Catalog Number	*03-085-1-*
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:
*HT 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 and thymidine. HT Medium is utilized in conjunction with HAT Medium which 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. The choice of medium used is dependent upon the current stage of the cloning process. The first cloning uses HAT medium and the second cloning uses HT medium. Any other subsequent clonings use a complete culture medium. For example, when the medium color is examined and untoward yellowing occurs, the plates are fed gradually with fresh HT medium as this further reduces the aminopterin from HAT and appears to enhance colony growth. Repeat cloning procedures are carried out until a stable and single hybridoma cell line is established. Stable Hybridomas yield >90% positive cultures upon recloning. When clones become stable, a 20 % Fetal Bovine Serum (FBS) level in the HT medium is reduced to half (i.e. to 10%), there is then a gradual reduction in HT level overall with the eventual and complete removal of the HT medium in its entirely. Remember that some clones are more sensitive to this HT weaning process than others.

Depending upon the specific protocol used, when screening for antibodies after a 7-10 day culture period, 100µl of HAT medium is removed from each well on day 5-6 and another 120µl of fresh HAT is added. Screening may then take place 2-3 days later. The transfer of Hybridomas from positive wells to 24 well-plates is carried out in HT medium. Culturing to near confluence and rescreening are carried out to ensure the steady secretion of antibody. Positive Hybridomas are recloned in HT Medium.

In sum, we see that initially since the cells have been previously grown in HAT medium and if selection against the unhybridized myeloma is completed, the HAT components are all removed from the medium with the result that the hybrids will die out. This result is mainly due to the fact that the hypoxanthine and thymidine are used up faster than the aminopterin and the cells have no recourse to the main or so-called salvage pathways for DNA synthesis. Maintaining the cells subsequently in HT medium allows the salvage pathways to operate. After splitting several times, the aminopterin concentration should be reduced sufficiently for the operation of the main pathway and thus the Hypoxanthine and Thymidine could in theory be omitted from the media. Sine this gradual weaning process sometimes proves to be very difficult with some hybridomas, some laboratories carry out subsequent operations in the HT medium. Before the cells can be switched to standard medium, they must be cultivated in the HT medium for up to two weeks (protocol-dependent) and often determined empirically. Due to numerous variations utilizing HAT and thus HT, most protocols are based on the individual researcher's experience and are not optimized for most hybridizations.

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-well 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.

Predominant Characteristics of HT 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. *Do 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.

Quality Control)

Test	Specifications
Appearance:	Clear Solution
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
HAT Supplement, Conc.,50X	03-080-1	-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