



Product Information

HYPOXANTHINE-AMINOPTERIN-THYMIDINE (HAT) MEDIA SUPPLEMENT [50X]

Product Number **H0262**

Storage Temperature -20°C

Product Description

The production of monoclonal antibodies involves the fusion of myeloma cells with spleen cells and the selection of hybridomas (cell hybrids) in culture utilizing hypoxanthine-aminopterin-thymidine (HAT) supplemented medium. Aminopterin blocks the synthesis of DNA by inhibiting dihydrofolate reductase³. Cells that lack the ability to utilize the salvage pathway for nucleotide synthesis are eliminated. Cells that possess hypoxanthine-guanine phosphoribosyl transferase (HPRTase) and thymidine kinase (TK) enzymes can utilize the salvage pathway if supplied with hypoxanthine and thymidine.^{1,2}

The purpose of the medium is to: (1) selectively kill unfused myeloma cells that are well adapted to tissue culture and would otherwise outgrow any hybridomas produced and (2) eliminate any myeloma-myeloma hybridomas that lack HPRTase. HPRTase positive spleen-spleen hybridomas, although not sensitive to aminopterin, are normally short-lived in culture.

After selection is complete (approximately 10-14 weeks), aminopterin is diluted from the culture by several passages of the cells in hypoxanthine-thymidine (HT)[Prod. No. H0137] supplemented medium (approximately 2-3 weeks) before transfer into normal hybridoma growth medium.

Components

Each vial contains:

Hypoxanthine 6.8 mg

Aminopterin 0.088 mg

Thymidine 1.94 mg

Precautions and Disclaimer

REAGENT: For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

HAT media supplement is supplied as a lyophilized, γ -irradiated powder for use in aseptic procedures. When reconstituted to 10 ml, each vial contains 5×10^{-3} M hypoxanthine, 2×10^{-5} M aminopterin and 8×10^{-4} M thymidine. When 10 ml of 50X concentrate are diluted to 500 ml with sterile tissue culture medium, the final concentrations of hypoxanthine, aminopterin and thymidine are 100 μ M, 0.4 μ M and 16 μ M, respectively.

Storage/Stability

Store at -20°C prior to and after reconstitution.

References

1. Freshney, R. I., *Culture of Animal Cells: A Manual of Basic Technique*, 3rd ed. (John Wiley & Sons, Inc., 1994) pp. 389-391.
2. Harlow, E. and D. Lane, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory, 1988) p. 280.
3. Kennett, R. H., *Meth. Enzymol.*, eds. W. B. Jakoby and I. H. Pastan (Academic Press, 1979) Vol. LVII, p. 352.

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