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 reductase. 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.

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 $5 \times 10^{-3}$ M hypoxanthine, $2 \times 10^{-5}$ M aminopterin and $8 \times 10^{-4}$ 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 µM, 0.4µM and 16 µM, respectively.

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

References

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