



## Product Information

### HYPOXANTHINE-THYMIDINE (HT) MEDIA SUPPLEMENT [50X]

Product Number **H0137**

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<sup>3</sup>. 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.<sup>1,2</sup>

The purpose of HAT medium [Prod. No. H0262] 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) supplemented medium (approximately 2-3 weeks) before transfer into normal hybridoma growth medium.

#### Components

Each vial contains:

Hypoxanthine 6.8 mg

Thymidine 1.94 mg

#### Precautions and Disclaimer

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

#### Preparation Instructions

HT media supplement is supplied as a lyophilized,  $\gamma$ -irradiated powder for use in aseptic procedures. When reconstituted to 10 ml, each vial contains  $5 \times 10^{-3}$  M hypoxanthine 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 and thymidine are 100  $\mu$ M and 16  $\mu$ M, respectively.

#### Storage/Stability

Store at -20 °C. After reconstitution, store at 2-8°C.

#### 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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