

fon:
 +49 (0)731 - 3608 123
 fax:
 +49 (0)731 - 3608 962
 eMail:
info@genaxxon.com
 internet:
www.genaxxon.com

HAT Supplement (50X)

Hypoxanthine, Aminopterin, Thymidine (HAT) Medium Supplement (50-times)

Product	Cat#	Package size
HAT supplement (50X)	C4210.0100	100mL
HAT supplement (50X)	C4210.1010	10x10mL

Description

The production of monoclonal antibodies involves the fusion of myeloma cells with spleen cells and the selection of hybridoma (cell hybrids) in cell culture utilizing hypoxanthine-aminopterin-thymidine (HAT) supplemented medium.

Aminopterin blocks the synthesis of DNA by inhibiting dihydrofolate reductase (3). 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: Selectively kill unfused myeloma cells that are well adapted to tissue culture and would otherwise outgrow any hybridomas produced and secondly 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.

Preparation

HAT medium supplement is supplied as a sterile ready-to-use formulation. Each bottle contains a $5 \times 10^{-3} \text{M}$ hypoxanthine, $2 \times 10^{-5} \text{M}$ aminopterin and $8 \times 10^{-4} \text{M}$ thymidine solution.

When 10mL of the 50X concentrate are diluted to 500mL with sterile tissue culture medium, the final concentrations of hypoxanthine, aminopterin and thymidine are 100 μM , 0.4 μM and 16 μM , respectively.

10mL of then supplied 50-times solution contains: Hypoxanthine: 68.0mg; Aminopterin: 0.88mg, Thymidine: 19.4mg.

Storage

HAT solution can be stored at +4°C for about 5 days.
 HAT solution can be stored at -20°C for about 2 years.

Prevent from repeated freeze-thaw cycles! Prepare aliquots for long term storage.

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.

Related products

Product	Cat#	Package size
HT supplement (50X)	C4211.0100	100mL
HT supplement (50X)	C4211.1010	10x10mL

For research use only.