

## HAT and HT

Monoclonal antibodies are produced by fusing myeloma cells with antigen-producing lymphocytes. A myeloma is a tumor of the bone marrow that can be adapted to grow permanently in cell culture. When fused with lymphocytes (spleen cells), the result is a cell with the ability to grow continually and produce large amounts of pure antibody. These very specific antibodies prove more effective than conventional drugs in fighting disease, since drugs attack both the foreign substance and the body's own cells. Monoclonal antibodies, however, attack the target molecule only, with little to no side effects.

Under normal conditions, there are two purine and pyrimidine synthetic pathways: the *de novo* pathway and salvage pathway. The *de novo* pathway synthesizes purines and pyrimidine from new material, while the salvage pathway converts old nucleotides to nucleotides that can be reused. Aminopterin, a folate antagonist, blocks the *de novo* biosynthesis of purines and pyrimidines by acting as a dihydrofolic acid analog; cells are prohibited from synthesizing nucleic acids and thus die. Only cells capable of utilizing the salvage pathway survive.

Dihydrofolate reductase is an enzyme that transfers hydrogen from NADP to dihydrofolate, yielding tetrahydrofolic acid, an essential vitamin cofactor in purine, thymidine, and methionine synthesis. Tetrahydrofolic acid is necessary for the *de novo* pathway.

HGPRT (hypoxanthine-guanine-phosphoribosyltransferase) is an enzyme that enables cells to synthesize purines through the salvage pathway using an extracellular source of hypoxanthine as a precursor. Some cells, including mutant myeloma cells, are unable to utilize this salvage pathway because they lack the required HGPRT enzyme. Because folic acid is necessary for purine biosynthesis and mutant cells are capable of only the *de novo* pathway, mutants cannot survive in the presence of aminopterin. Successfully fused hybridoma cells, however, can synthesize DNA through the salvage pathway, thanks to the HGPRT enzyme supplied by the spleen cell. The hypoxanthine and thymidine supply preformed purines and pyrimidines to the salvage pathway for DNA synthesis.

HAT media selects for only those successfully fused hybridoma cells. Once all mutant self-fused myelomas or un-fused myeloma cells have been eradicated from the media and aminopterin is no longer needed, the cells may be cultured with HT media.

HT is a "rescue media", providing preformed purines and a pyrimidine to overcome the effects of residual intracellular Aminopterin. Once the *de novo* biosynthesis pathway for nucleosides has been reestablished, HT is no longer needed in the culture medium.

Product Description	Catalog No.	Size
HAT 50X Solution	25-046-CI	1 x 100 mL
HT 50X Solution	25-047-CI	1 x 100 mL