

## Technical Information

### HAT Medium supplement 50X, Liquid

w/ 680.5 mg/litre Hypoxanthine, 8.8 mg/litre Aminopterin and 193.8 mg/litre Thymidine in Phosphate Buffered Saline Sterile filtered Cell Culture Tested

### Product Code: TCL1072

#### Application:-

Monoclonal antibodies are produced by hybridoma technology in which a non-secreting myeloma cell line is fused with an antibody-producing B-lymphocyte. After fusion, the proportion of viable hybrids is low. Hence, selective media are required to favor the survival of hybrids at the expense of the parental cells. Hybrids are most frequently selected with the HAT system.

HAT supplement contains Hypoxanthine, Aminopterin and Thymidine and is used for the preparation of selection medium for hybridoma. The myeloma cell is deficient in enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) or thymidine kinase (TK), and cannot survive in selection medium containing hypoxanthine, aminopterin and thymidine. Any unfused B-lymphocytes from the spleen cannot survive in culture for more than a few days. It is essential for B-cell-myeloma hybrids to contain the genetic information from both parent cells to enable them to survive in the HAT selection system. They can be cultured indefinitely and will produce unlimited quantities of antibody.

After selection of the hybrid cells, the aminopterin from the medium is diluted by several passages in hypoxanthine-thymidine (HT) supplemented medium for about 2-3 weeks. The hybrids are then transferred to a hybridoma growth medium.

TCL1072 is a sterile filtered 50X HAT medium supplement containing 680.5 mg/litre Hypoxanthine, 8.8 mg/litre Aminopterin and 193.8 mg/litre Thymidine in Phosphate Buffered Saline.

### Methodology

1. Aseptically transfer 10 ml of the 50X HAT medium supplement to 500ml of sterile medium.

2. Tightly cap the media bottle and mix gently to ensure proper mixing.

Note: Do not mix vigorously as it may lead to formation of foam.

3. The final concentrations of hypoxanthine, aminopterin and thymidine in 500ml media will be 100mM, 0.4mM and 16mM respectively.

### Quality control

#### Appearance

Colorless, clear solution

#### pH

8.90 to 9.50

#### Cell Culture Test

Passes

#### Sterility

No bacterial or fungal growth is observed after 14 days of incubation, as per USP specifications

#### Endotoxin Content

NMT 1EU/ml

### Storage and Shelf Life

Store the product at -20°C. Avoid repeated freezethaw cycles.

Use before expiry date given on the product label.

### Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
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