Hybridoma Supplement

#GTC07.0050 (100ml)
(FOR RESEARCH ONLY)

Product: Hybridoma Supplement is a chemically defined serum-free growth promoting supplement, suitable for fusion, selection and cloning of hybridoma cells. The supplement consists of ethanolamine, hydrocortisone, retinoic acid, linoleic acid, insulin and other growth factors. Since Hybridoma Supplement can be added directly to the culture medium, there is no need for the use of feeder cells, which includes risks of overgrowth, contamination, competition for nutrients, and variations in growth factor concentrations. Hybridoma Supplement can be used with serum-free culture media and its low protein content facilitates the purification of monoclonal antibodies from the medium.

Quantity: #GTC07.0050 comprises 50ml of a 10x concentrated solution of Hybridoma Supplement.

Applications: Cell Culture. Serum-free media. Monoclonal Antibodies.

Appearance: Clear pale reddish solution.

Specifications:

pH:	6.8-7.6
Sterility:	sterile

Storage: Store at -20°C for up to 1.5 years.

Prior to use:

Thaw Hybridoma Supplement by placing the vial in a 37°C water bath. During thawing, occasionally swirl gently. After thawing, do not leave at 37°. Alternatively, thaw by storing it overnight in a refrigerator (+2°C to +8°C). Hybridoma Supplement can be re-frozen, however, this should be avoided as it contains highly sensitive components. If this cannot be avoided, aseptically prepare aliquots of convenient volume and store at -20°C.

Fusion

Use Hybridoma Supplement to boost hybridoma yield during HAT selection and increase the number of antibody-producing clones.

1. Carry out fusion of splenocytes and myelomas according to normal protocols and centrifuge cells to remove polyethylene glycol.
2. Resuspend hybridomas in hybridoma HAT selection medium containing 5% to 10% Hybridoma Supplement. A density of $5x10^4$ to $5x10^5$ spleen cells per ml (initial amount used for fusion) is required for the distribution into 96-well culture plates.
3. *[Alternatively]* Instead of distribution into 96-well culture plates, Hybridomas may be resuspended in, half of the desired volume for plating, of fresh hybridoma HAT selection medium. After 18-24 hours, add an equal volume of hybridoma growth medium containing double concentration of HAT and 5% to 10% Hybridoma Supplement.
4. After 10 days of proliferation, colonies will be visible by eye, and the supernatant may be assayed for the presence of antibody.
5. Positive clones may be expanded in medium containing 5% to 10% Hybridoma Supplement.

Cloning

Use Hybridoma Supplement to improve the cloning efficiency of hybridomas

1. Cultivate hybridomas in cell-specific hybridoma growth medium containing 10% Hybridoma Supplement until cells reach logarithmic phase (~$5x10^5$ cells/ml)
2. Count cells and dilute (approximately 100.000 times) in fresh medium containing 10% to 15% serum, to a final density of 5 cells/ml
3. Distribute into wells of a 96-well tissue culture plate (200µl/well) and allow cells to grow for 10 to 14 days (without refeeding).
4. Check for macroscopic colonies and assay the supernatant of wells containing single colonies for antibody presence.
5. Aid expansion to 24-well plates by culturing hybridomas in growth medium containing 5% to 10% Hybridoma Supplement.

Monoclonal Antibody Production

Use Hybridoma Supplement to enhance the growth of hybridomas under serum-free conditions

1. Add 5% to 10% Hybridoma Supplement to the serum-free culture medium and proceed with monoclonal antibody production according to normal protocols.