Product Information

Hybridoma Supplement
Cat. No. HCS-F (50 ml)

General Information

Hybridoma cells result from fusion of an antibody producing B-cell from the immune system with a tumor cell. To support hybridoma development and achieve an optimal cell density, as well as cloning efficiency, growth factors and serum (10 % to 20 %) are required. Hybridoma Supplement contains insulin, ethanolamine, hydrocortisone, retinoic acid, linoleic acid, and other growth promoting factors. As supplement to culture medium it supports the growth of hybridoma in a manner feeder cells have been used before. The disadvantages occurring by the use of feeder cells may include: overgrowth of newly formed hybridomas, source of contamination, competition for nutrients, and variations in growth factor concentrations. Hybridoma Supplement can be used in culture medium under serum-free conditions. The low protein content facilitates the isolation and purification of produced antibodies. Hybridoma Supplement is provided as a 10x concentrate.

Applications:

- Improvement of Hybridoma Growth after fusion.
- Improvement of cloning efficiency of hybridomas.
- Production of Monoclonal Antibodies under serum-free conditions.

Product Specifications

Appearance	Clear red orange solution
Storage	Store at ≤-15°C.
Shipping conditions	Frozen (dry ice)
Working concentration	Recommended working concentration: 5 % to 10 %

Instructions for Use

Thawing:

To thaw the frozen Hybridoma Supplement, place the vial in a 37°C water bath or store it overnight in the fridge. During thawing in a water bath move the vial gently and do not leave at 37°C after thawing.

Hybridoma Growth after Fusion:

HCS improves the yield of hybridomas during HAT selection and enhances the number of antibody-producing clones.

1. Perform fusion of splenocytes and myelomas according to established protocols and centrifuge cells to remove the polyethylene glycol.
2. Resuspend the newly fused hybridomas in hybridoma HAT selection medium, i. e. complete IMDM or RPMI (10 % to 20 % serum, 0.1 mM 2-mercaptoethanol and HAT) containing 5 % to 10 % Hybridoma Supplement. A density of $5x10^4$ to $5x10^5$ splenocytes per ml for distribution into 96 well tissue culture plates is required.

   Alternatively, the newly fused hybridomas may be resuspend in the same medium in half the final desired volume to be plated on tissue culture treated surface. After 18 to 24 hours an equal volume of hybridoma growth medium containing 5 – 10% HCS and two times the final concentration of HAT is added to the plates.
3. After ten days of proliferation (no refeeding of the cultures is necessary), the colonies will be visible by eye and the supernatant fluids may be assayed for antibody.
4. Antibody positive hybridomas may be expanded in medium containing 5 % to 10 % Hybridoma Supplement.

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Hybridoma Cloning:

HCF improves the cloning efficiency of hybridomas.

1. Cultivate the hybridomas in prepared cell-specific hybridoma growth medium containing 10 % Hybridoma Supplement until cells reach the logarithmic phase (approximately $5x10^5$ cells/ml).
2. Count the cells and dilute them in the same medium containing at least 10 % to 15 % serum to a density of 5 cells per ml.
3. Distribute 0.2 ml of the cell suspension to each well of a 96 well tissue culture plate.
4. Allow the cells to grow for 10 to 14 days (no refeeding is necessary) and check for macroscopic colonies.
5. Assay the supernatant of wells containing single colonies for antibody. Aid Expansions to 24 well plates by culturing the hybridomas in growth medium containing 5 % to 10 % Hybridoma Supplement.

Production of Monoclonal Antibodies under Serum-fee Conditions:

Hybridoma Supplement enhances the growth of hybridomas under serum free conditions.
5 % to 10 % Hybridoma Supplement should be added to the culture medium.

Precautions and Disclaimer

This product is for research use and further manufacturing only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Help Needed?

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (techservice@capricorn-scientific.com) or phone (+49 6424 944640).