Corning® hybrigro SF™ Medium

Animal-free, Serum-free Defined Medium with Corning® glutagro™ Supplement

Protocol

CORNING

Adaptation and Weaning Techniques in Corning® hybrigro SF™ Medium

Direct adaption or weaning techniques can be used to adapt cells to Corning® hybrigro SF™ medium (Corning Cat. No. 40-215-CV). While most hybridoma cells do not require weaning, this method is recommended as a precaution due to possibly significant differences in formulation between the current media used and hybrigro SF medium.

Important factors leading to successful adaptation include:

- Cells must be in the mid-logarithmic phase of growth with viability >90% prior to adaptation.
- Higher seeding density than normal is recommended to increase conditioned medium carry over the early period of adaptation.

Direct Adaptation

1. A seeding density of double the normal seeding density for the cell line is suggested for the first passage.
2. Subculture cells when the cell density is 2×10^6 to 3×10^6 cells/mL.
3. For passage numbers 2 to 4, subculture the cells to a viable cell density of 2×10^5 cells/mL in fresh, serum-free medium.

Weaning

Gradually change the medium over 2 to 3 passages by blending the two media 50:50 for the first passage and then 25:75 for the second. Seed cells at ~5 x 10^5 cells/mL.

Maintenance in Corning hybrigro SF Medium

Hybridoma cultures should be split into fresh, serum-free medium every 3 to 4 days and seeded at 1 to 2 x 10^5 cells/mL to maintain optimal growth and productivity.

Cryopreservation

1. Gently pellet mid-log phase cells for freezing. Remove supernatant and resuspend in a volume of cryopreservation medium (e.g., 500 µL conditioned medium: 500 µL fresh medium and 50 to 100 µL DMSO) to obtain a final cell density of 1 to 5 x 10^6 cells/mL.
2. Dispense into cryovials and freeze following standard procedures.

Thawing

1. Once the last amount of ice thaws, quickly transfer the contents of the vial so the cells are seeded at 4 to 5 x 10^5 cells/mL in fresh serum-free medium. If using centrifuge, take care to use low speed as cells are extremely fragile following freezing.
2. Resume normal maintenance schedule. Monitor cell growth and viability.

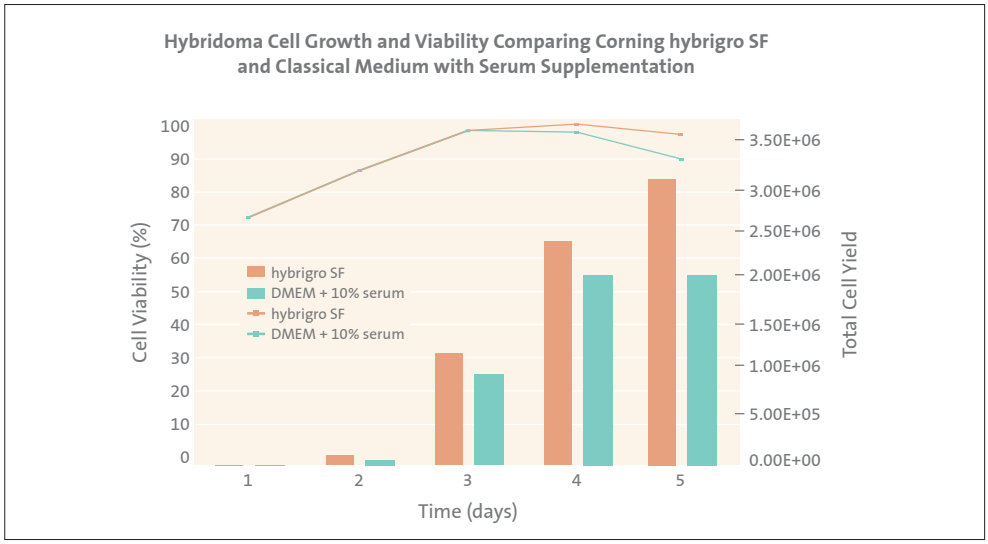

Figure 1. Comparison of Corning® hybrigro SF™ medium versus standard serum-supplemented medium using AE1 (ATCC® HB-72™) hybridoma cell line. Growth with hybrigro SF medium is superior and exhibits enhanced viability, as compared to a standard serum-supplemented medium.

Note: hybrigro SF medium will not grow cholesterol-auxotrophic myeloma-derived hybridomas (e.g., NS0, NS1, and P3X63Ag8.653) without further supplementation. Supplementation with a solubilized preparation of cholesterol is required for these cell line derivatives.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

Beginning-to-end Solutions for

Cell Culture

www.corning.com/lifesciences/solutions

At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.

For additional product or technical information, please visit www.corning.com/lifesciences/media or call 1.800.235.5476.

Mediatech, Inc.
A Corning Subsidiary
9345 Discovery Boulevard
Manassas, VA 20109
t 800.235.5476
t 703.471.5955
f 703.467.9851
www.corning.com/lifesciences/media

CORNING | FALCON® AXYGEN® GOSSELIN™ PYREX®