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ProductInformation

HYBRIDOMA MEDIUM
ANIMAL COMPONENT- FREE
Without L-Glutamine

Product Code **H 4409**
Storage Temperature 2-8 °C

Synonyms: Hybridoma Medium AF

Product Description
Hybridoma Medium, Animal Component-Free has been developed to specifically meet the demands of the life science and biotechnology industries. This medium supports high viable cell densities and high antibody productivity over extended culture periods of 60 days or greater. The formulation is suitable for use in cloning, fusion, and therapeutic applications. It further minimizes protein levels in downstream production that could interfere with antibody purification. The elimination of animal-derived components reduces the incidence of performance variability in the medium and eliminates safety risks due to adventitious agents associated with these components.

Intended Use
For R&D use only. Not for drug, household or other use.

Components
The proprietary formulation includes inorganic salts, essential and non-essential amino acids, vitamins, sodium bicarbonate, HEPES, trace elements, fatty acids, and other organics. Recombinant human insulin (4 mg/L) is the only source of protein in the medium. The medium does not contain L-glutamine, antibiotics, and phenol red.

Preparation Instructions
This medium is supplied as a sterile 1X liquid. Aseptically add 50 ml of 200 mM L-glutamine (Product Code: G 7513) to each liter of medium prior to use.

Storage/Stability
This medium is stable, when stored at 2-8 °C and protected from light, until the date indicated on the label.

Procedure
Thawing Frozen Cultures
1. Rapidly thaw a 1-ml vial of cryopreserved cells in a 37 °C water bath.
2. Transfer thawed cells to a 15-ml conical centrifuge tube containing 3 ml of Hybridoma Medium AF.
3. Mix well by gently inverting or swirling the tube.
4. Determine the viable cell density by trypan blue exclusion (Product Code: T 8154).
5. Centrifuge at 200 x g for 5 minutes.
6. Remove supernatant and re-suspend cells in 2-5 ml of fresh medium.
7. Transfer to a cell culture T-flask and add sufficient medium to bring cells to a density of 2×10^5 viable cells/ml.
8. Place the T-flask in a humidified incubator at 37 °C and 5% CO_2.

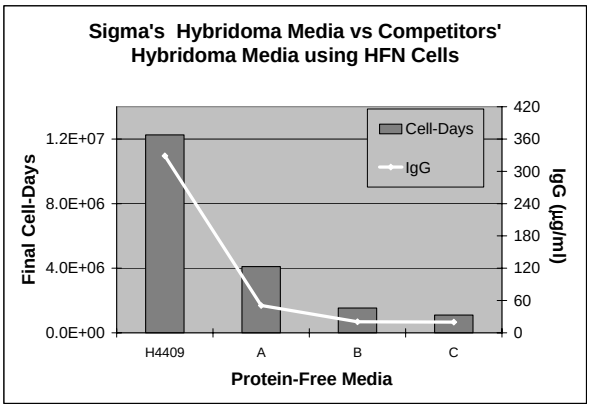
Adaptation to Hybridoma Medium AF
Most hybridoma cells do not require weaning from serum-containing medium prior to inoculation in Hybridoma Medium AF. Should direct inoculation be unsuccessful, cells should be cultured in basal medium containing 10% FBS to a cell density of 5×10^5 to 1×10^6 cells/ml. Next, harvest and re-seed the cells at 1×10^5 cells/ml in the basal medium containing 2% FBS. At subsequent passage, split the cells into a 50:50 ratio of the basal medium with 2% FBS and Hybridoma Medium AF. Continue to reduce the ratio of serum-containing medium: Hybridoma Medium AF (25:75) at the subsequent passage and finally to 100% Hybridoma Medium AF (0:100). If a cell line is cholesterol-dependent, it may be necessary to add a source of cholesterol (Product Code: C 1231) at a final concentration of 2-5 mg/ml in Hybridoma Medium AF.
Maintenance of Established Cultures
Hybridoma cells should be passaged frequently to prevent cells from reaching excessive densities in T-flask. Generally, they are plated at 1×10^6 cells/ml. Both maximum and minimum densities may vary from cell line to cell line. Most hybridoma cell lines should be passaged 3 times per week, but some slow-growing cell lines may require a more extended culture period between passages.
Cryopreservation
Pellet cells grown in Hybridoma Medium AF at 200 x g for 5 minutes. Remove the supernatant. Re-suspend in Serum-Free Cell Freezing Medium (Product Code:

C 6295) at a density of 1 x 10^6 to 5 x 10^6 cells/ml. Dispense aliquots to freezer vials and freeze in liquid nitrogen (1 °C decrease per minute).

Product Profile

Sigma's Hybridoma Medium AF (Product Code: H 4409) shows excellent cell growth and antibody productivity. [4] For these studies, HFN 7.1 cells (ATCC® CRL-1606) grown in DME/F12 medium containing 10% FBS and frozen in 1-ml aliquots of 10% FBS were used. The cells were thawed, transferred into DME/F12 medium containing 10% FBS, and adapted for growth over ten days in each of the protein-free media products (see Procedure: Adaptation to Hybridoma Medium AF).

Comparison of Sigma's Hybridoma Medium AF with protein-free hybridoma media from three competitors (A, B, C) demonstrates that the Sigma product ranks at the top of commercially available hybridoma media. The figure shows the average growth and productivity resulting from three experiments. Final "Cell-Days" is the integral of the area below the plot of viable cells vs time as a measurement of the overall supporting capacity of the medium.



References

1. Morrow, K.J., Monoclonal antibody production techniques. Gen. Eng. News, **20(14),** 21 (2000).
2. Wrotnowski, C., Cell culture media trends mirror bioindustry. Gen. Eng. News, **20(8),** 8, (2000).
3. Harlow, E. and Lane, D., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory, New York, 1988).
4. Peppers, S. et al., Performance-optimized hybridoma medium: replacing serum and other animal-derived components. LifeScience Quarterly, **2(2),** 6-10, (2001). [LifeScience Quarterly is a newsletter distributed by Sigma-Aldrich Corporation]

Precautions and Disclaimer

MSDS is available upon request or at www.sigma-aldrich.com. ATCC is a registered trademark of American Type Culture Collection.

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