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ProductInformation

HYBRIDOMA MEDIUM
ANIMAL COMPONENT- FREE
Without L-Glutamine and Sodium Bicarbonate

Product Code **H 8784**
Storage Temperature 2-8 °C

Synonym: 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 more. The formulation is suitable for use in cloning, fusion, and antibody production for 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

Powdered media are extremely hygroscopic and should be protected from atmospheric moisture. The entire contents of each package should be used immediately after opening. Preparing a concentrated solution of medium is not recommended as precipitates may form.

Supplements can be added prior to filtration or introduced aseptically to sterile medium. The nature of the supplement may affect storage conditions and shelf life of the medium.

1. Measure out 85% of final required volume of water. Water temperature should be 15-20 °C.
2. While gently stirring the water, add the powdered medium. Stir for 10-15 minutes.

   Note: Do NOT heat. Contents may not dissolve completely until step 3.
3. Add 1.2 g sodium bicarbonate or 16 ml of sodium bicarbonate solution (7.5% w/v) for each liter of final volume of medium being prepared. Continue stirring until completely dissolved.
4. To the solution in step 3, add 1.46 g of L-glutamine or 50 ml of L-glutamine solution (200 mM) for each liter of final volume of medium being prepared. Stir until completely dissolved. If preferred, L-glutamine solution may be added aseptically after medium filtration, but with the final volume in step 6 reduced accordingly.
5. While stirring, adjust the pH of the medium to 0.1-0.3 pH units below the desired pH, since it may rise during filtration. The use of 1N HCl or 1N NaOH is recommended.
6. Add additional water to bring the solution to final volume.
7. Sterilize immediately by filtration using a membrane with a porosity of 0.22 micron.
8. Aseptically dispense medium into sterile container.

Storage/Stability

This medium is stable when stored at 2-8 °C under dry conditions until the date indicated on the label. Store liquid medium prepared from the powdered medium at 2-8 °C in the dark. Note that the nature of supplements added may affect storage conditions and shelf life of the liquid medium.

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 resuspend cells in 2-5 ml of fresh medium.
7. Transfer to a cell culture T-flask and add sufficient medium to bring the cells to a density of 2 x 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 x 10^5 to 1 x 10^6 cells/ml. Next, harvest and re-seed the cells at 1 x 10^5 cells/ml in the basal medium containing 2% FBS. At subsequent passage, split the cells into a 50:50 mixture of the basal medium with 2% FBS and Hybridoma Medium AF. Continue to reduce the ratio of serum-containing medium to 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/L in Hybridoma Medium AF.

Maintenance of Established Cultures

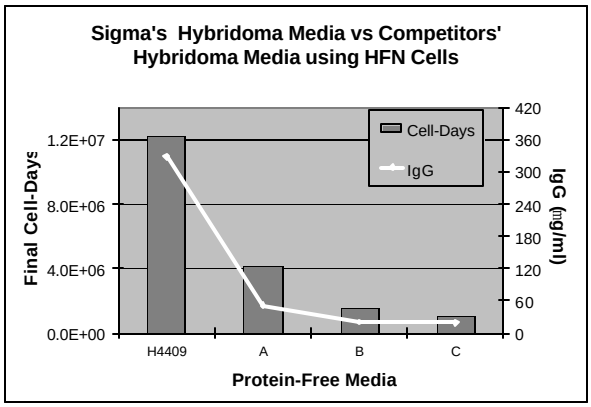
Hybridoma cells should be passaged frequently to prevent cells from reaching excessive densities in T-flasks. Generally, they are plated at 1 x 10^6 cells/ml. Both maximum and minimum densities may vary from cell line to cell line. Most hybridoma cell lines should be passaged 3 times per week, but some 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. Resuspend 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 Codes H 8784 for powdered medium or H 4409 for liquid medium) 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 versus 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.

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