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Product Information

EX-CELL® Hybridoma Medium

Catalog Number **H4281**
Storage Temperature 2–8 °C

Product Description

EX-CELL® Hybridoma Medium 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 and fusion applications. It further minimizes protein levels in downstream production that could interfere with antibody purification. The elimination of serum reduces performance variability in the medium and eliminates safety risks associated with possible adventitious agents in serum.

The proprietary formulation includes inorganic salts, essential and non-essential amino acids, vitamins, sodium bicarbonate, HEPES, trace elements, fatty acids, and other organics. It contains low concentrations of recombinant human insulin, bovine serum albumin, and human transferrin (source tested negative for HIV antibody and HbsAG). The medium does not contain L-glutamine, antibiotics, antimycotics, and phenol red.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

This medium is supplied as a sterile 1× liquid. Supplement the medium with L-glutamine to a final concentration of 10 mM by aseptically adding 50 ml of 200 mM L-glutamine solution, Catalog Number G7513, to each liter of medium.

Storage/Stability

This medium is stable when stored at 2–8 °C and protected from light until the date indicated on the label.

Procedures

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 EX-CELL Hybridoma Medium.
3. Mix well by gently inverting or swirling the tube.
4. Determine the viable cell density by trypan blue exclusion, Catalog Number T8154.
5. Centrifuge at 200 × *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 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 EX-CELL Hybridoma Medium

Most hybridoma cells do not require weaning from serum-containing medium prior to inoculation in EX-CELL Hybridoma Medium. Generally, initial cell inoculation is $2–5 \times 10^5$ cells/ml. Cells should be cultured in basal medium containing 10% FBS to a cell density of 5×10^5 to 1×10^6 cells/ml if direct inoculation is not successful. 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 basal medium with 2% FBS and EX-CELL Hybridoma Medium (50:50). Continue to reduce the ratio of serum-containing medium to EX-CELL Hybridoma Medium (25:75) at the subsequent passage and then to 100% EX-CELL Hybridoma Medium. If a cell line is cholesterol-dependent, it may be necessary to add a source of cholesterol, Catalog Numbers C1231 or C3045, at a final concentration of 2–5 mg/ml in EX-CELL Hybridoma Medium.

Maintenance of Established Cultures

Hybridoma cells should be passaged frequently to prevent cells from reaching excessive densities in the T-flask. Generally, they are plated at 1×10^6 cells/ml. Both maximum and minimum densities may vary with each 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

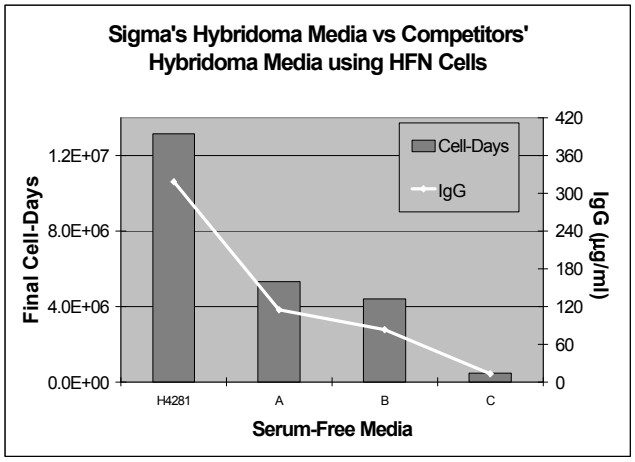
Pellet cells grown in EX-CELL Hybridoma Medium at $200 \times g$ for 5 minutes. Remove the supernatant. Resuspend in Serum-Free Cell Freezing Medium, Catalog Number C6295, at a density of $1–5 \times 10^6$ cells/ml. Dispense aliquots to freezer vials and freeze in liquid nitrogen (1 °C decrease per minute).

Results

EX-CELL Hybridoma Medium shows excellent cell growth and antibody productivity.[4] For these studies, HFN 7.1 cells (Catalog Number 89062001) 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 serum-free media products (see Procedures, Adaptation to EX-CELL Hybridoma Medium).

Comparison of EX-CELL Hybridoma Medium with serum-free hybridoma media from three competitors (A, B, C) demonstrates the Sigma product ranks at the top of commercially available hybridoma media. Figure 1 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.

Figure 1.



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