Product Information

Hybridoma-Max, Protein-free Hybridoma Medium, with L-Glutamine

Cat. No.: S0500-520 Volume: 500 ml

Product Description

Hybridoma-Max is a protein-free, ready-to-use medium that contains no polypeptide growth or attachment factors, or mediators that may complicate downstream processing and final product purification. Hybridoma-Max also performs well as serum-supplemented media for monoclonal antibody production and also may be used as a growth medium.

Product Specifications

pH	7.0 – 7.5
Endotoxin	≤ 1 EU/ml
Cell Culture	Tested
Sterility	Tested
Storage	+2°C to +8°C

Important Information

- Hybridoma-Max requires supplementation with a cholesterol supplement or some other source of cholesterol for growth of cholesterol-dependent cell lines (e.g., NS0 and derivatives).
- Hybridoma-Max does not contain insulin or transferrin.
- This medium does not contain a surfactant. If used for agitated suspension culture, supplement with 0.1 % Pluronic® F-68.
- In most instances, antibiotics are neither necessary nor advised. However, where antibiotics are required, most general antibiotics are compatible with Hybridoma-Max, including penicillin/streptomycin, gentamicin, anti-PPLO, linocin, and amphotericin B. Do not use kanamycin sulfates or neomycin sulfates.

Culture Conditions

Culture Type:	Suspension
Culture Vessels:	Shake flasks, roller bottles or bioreactor.
Temperature Range:	36°C to 38°C
Incubator Atmosphere:	Humidified atmosphere of 5 % to10 % CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Recovery

1. Rapidly thaw (< 1 minute) frozen cells in a +37°C water bath.
2. Transfer the entire contents of the cryo vial into a tissue culture flask containing 30 ml pre-warmed Hybridoma-Max without antibiotics.
3. Incubate at +37°C in a humidified atmosphere of 5 % CO_2 in air.
4. Subculture cells 3 to 5 days post thaw.

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Adapt Hybridoma Cells to Hybridoma-Max

Successful adaptation will depend upon the particular hybridoma cell line and the culture conditions employed. We recommended that backup cultures in the original medium be maintained until success with the new medium has been achieved.

Note: It is critical that cell viability be at least 90 % and cells be in the mid-logarithmic phase of growth prior to adaptation.

Direct Adaptation

1. Subculture hybridoma cells grown in conventional medium with 5 to 10 % serum or other serum-free medium into pre-warmed Hybridoma-Max. During the adaptation procedure seeding density should be double the normal seeding density for the cell line.
2. Monitor cell growth until the viable cell density reaches 1×10^6 viable cells/ml. Subculture the cells to a viable cell density of $1–2 \times 10^5$ viable cells/ml in fresh pre-warmed Hybridoma-Max.
3. Continue to monitor and passage cells for 3 to 5 passages until consistent growth is achieved.

Note: If suboptimal performance is observed over 3 to 5 passages using the direct adaptation method, use the sequential adaptation method.

Sequential Adaptation

1. Subculture hybridoma cells grown in conventional medium with 5 to 10 % serum or other serum-free medium into a 25:75 ratio of fresh Hybridoma-Max to the original media. During the adaptation procedure seed at double the normal seeding density.
2. Monitor cell growth until the viable cell density reaches 1×10^6 viable cells/ml. Subculture cells (dilute to $1–2 \times 10^5$ viable cells/ml) into stepwise increasing ratios of fresh Hybridoma-Max to original medium with each subsequent passage (50:50, 75:25, 90:10 followed by 100 Hybridoma-Max). Multiple passages at each step may be required.
3. Continue to monitor and passage cells until consistent growth is achieved. After several passages of consistent growth and viability in 100 % complete Hybridoma-Max the culture is considered to be adapted.

Cryopreservation

1. Prepare the desired quantity of cells in a tissue culture flask, harvesting in mid-log phase of growth with viability > 90 %. Reserve the conditioned medium to prepare cryopreservation medium.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of $0.5–1 \times 10^7$ cells/ml.
3. Prepare the required volume of cryopreservation medium of 92.5 % medium (50:50 ratio of fresh to conditioned media) + 7.5 % DMSO on the day of intended use. Filter sterilize and store at 4°C until use. Important: Conditioned medium should be obtained from a high viability, mid-log culture of cells.
4. Harvest cells by centrifugation at 100 × g for 5 to 10 minutes. Re-suspend the pellet in the pre-determined volume of +4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryo vials according to the manufacturer's specifications (i.e., 1 ml in a 2-ml cryo vial).
6. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen (vapor phase); storage at –200°C to –125°C is recommended.

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Precautions and Disclaimer

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

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