UAB "LT Biotech"

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HybridomaFirst
Serum-free Medium for Hybridoma Cells, with L-Glutamine, sterile-filtered
Cat. No.: FASFM-500ML (500 ml)

General Information

HybridomaFirst is a defined medium to cultivate hydridoma cells under serum free conditions. The medium is intended as a monoclonal antibody production medium, although it also has been used to grow a variety of hybridoma cell lines.

Applications

- MAb production
- Cell line cultivation

Features

- Contains 4 mM L-glutamine
- Contains Iron Citrate
- Free from animal components
- Contains yeast extract to enrich the medium for maximum performance

Appearance	Clear yellow to red liquid
Storage and shelf life	Store at +2°C to +8°C. Once opened store at 4°C and use within 6-8 weeks.
Shipping conditions	Ambient

Formulation

This formulation is our proprietary composition and has no counterparts either in its composition, or in its action.

Important Information

- The medium does not contain insulin or transferrin. Supplementation with insulin (e.g. 5 mg/l insulin) may further improve the performance of this medium.
- HybridomaFirst requires supplementation with a cholesterol supplement or some other source of cholesterol for growth of cholesterol-dependent cell lines (e.g., NS0 and derivatives).
- This medium does not contain a surfactant. If used for agitated suspension culture, supplement with 1 g/l Pluronic® F-68.
- In most instances, antibiotics are neither necessary nor advised. However, where antibiotics are required, most general antibiotics are compatible with HybridomaFirst, including penicillin/streptomycin, gentamicin, anti-PPLO, linocin, and amphotericin B. Do not use kanamycin sulfates or neomycin sulfates.

Culture Conditions

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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 of cells

1. Rapidly thaw (< 1 minute) frozen cells in a +37°C water bath.
2. Transfer the entire contents of the cryovial into a tissue culture flask containing 30 ml prewarmed HybridomaFirst 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.

Adapt Hybridoma Cells to HybridomaFirst

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 prewarmed HybridomaFirst. 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 × 106 viable cells/ml. Subculture the cells to a viable cell density of 1–2 × 105 viable cells/ml in fresh prewarmed HybridomaFirst.
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 HybridomaFirst 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 × 10^5 viable cells/ml) into stepwise increasing ratios of fresh HybridomaFirst to original medium with each

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subsequent passage (50:50, 75:25, 90:10 followed by 100 HybridomaFirst). 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 HybridomaFirst the culture is considered to be adapted.

Cryopreservation of Cells

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 × 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. Resuspend the pellet in the pre-determined volume of +4°C cryopreservation medium.

5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1 ml in a 2-ml cryovial).

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.