Neo Hybridom, protein-free hybridoma medium, w/o L-Glutamine, w/o Phenol Red

#Cat: NB-58-0084 Size: 500ml

General Information

Neo Hybridom 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. Neo Hybridom also performs well as serum-supplemented media for monoclonal antibody production and also may be used as a growth medium.

Product Specifications

Appearance	Clear liquid
Storage and shelf life	Store at +2°C to +8°C, protected from light 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

- Neo Hybridom requires supplementation with a cholesterol supplement or some other source of cholesterol for growth of cholesterol-dependent cell lines (e.g., NS0 and derivatives).
- Neo Hybridom 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 Neo Hybridom, 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 % to 10 % 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 Neo Hybridom 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 Neo Hybridom

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 Neo Hybridom. 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 prewarmed Neo Hybridom.
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 Neo Hybridom 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 Neo Hybridom to original medium with each subsequent passage (50:50, 75:25, 90:10 followed by 100 % Neo Hybridom). 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 Neo Hybridom 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 × 107 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.

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Precautions and Disclaimer

This product is for research use and further manufacturing only.

Help Needed?

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (info@neo-biotech.com) or phone (+33 9 77 40 09 09).