FT-826430

Hybridokine

Product Description

Features: **A hybridoma growth medium supplement**

- contains a mixture of growth factors for the culture of hybridoma
- an ideal replacement for feeder cell layers (no risk of contamination)
- improves the selection and then cloning efficiency of antibody producing hydridomas, and their stabilization (increases the number of cells which secrete antibody, increases the cell viability including under stress conditions such as defrosting)
- convenient: dilute 1:10 with growth medium and add to hybridoma cells
  recommended for use at a final concentration of 2.5 % (v/v) in medium

Catalog number: UP826430, 12.5ml
Form: sterile and lyophilized
Storage: +4°C, stable 6 months when stored lyophilised at 0–5°C (K)
two years when stored lyophilised at –20°C

Scientific and Technical Information

Hybridoma and their growth factors

- **Myeloma (and their hybridoma)** are tumoral cell lines that have the ability to proliferate indefinitely. One important application is the production of monoclonal antibodies that are recovered in the cell culture medium. Myeloma are fused with splenocytes from immunized animals to yield hydridoma which combine immortality and specific antibody secretion. This require specific culture conditions, i.e. most require growth factors to maintain optimal growth.

- One key step in the hybridoma obtention is the **cloning** and clones **stabilization**. Cloning consists to obtain a culture from a unique cell. This critical step should be done rapidly after parent cells were fused. It is recommended to repeat cloning from time to time to avoid the development of non interesting cells that may appear and overcome the growth of the right cell line (producing the desired specific antibody). To promote optimal growth of the hydridoma, cell culture media should be supplemented with cytokines and Foetal Calf Serum (FCS). However protein concentration should be reduced when interfering with further treatment, like antibody purification.

- Many ways attempted to improve cell culture media and supplements. **Feeders** obtained from various cell layers **(Macrophages, splenocytes...)** are frequently used in hybridoma cloning, but their preparation is a hassle and gives tedious or variable results. **Hybridokine** is a break throught alternative to that purpose.

Hybridokine

- The 'Hybridokine' (**Hybridoma cloning enhancing factor**) is obtained and purified from media of continuous culture of a human tumoral cell line (ES1, a strain of Ewing sarcoma). It contains a potent growth factor that allows and accelerates the growth of cells, even a unique cell (making it ideal for cloning procedures). It is a mixture whose main activity relay on a undefined cytokine, and Interleukine 6. Although its nature is not well defined, the biological activity is perfectly controlled. It favors the growth of hydridoma of splenocytes and myeloma, as well various other cell lines.

- In monoclonal Ab development and production, Hybridokine increases the cell viability, hence it enhances the number of antibody producing clones during hybridoma selection after splenocytes/myeloma fusion and during hybridoma cloning procedures. It helps cells under stress conditions such as during freezing/defrosting, improving hydridoma recovery and growth when used before and after cryo-storage. Finally, Hybridokine allows to produce up 5 fold more colonies per 96-well plate than medium without growth supplement, and +10 to +50% more colonies than with (splenocytes, peritonela cells, thymocytes) feeder layers.
  Hybridokine is an efficient and economical growth promotor for many strains: mouse / mouse (X63, SP2/0, NS1); rat / rat (Y3); mouse / human ; mouse / rat; Hybridoma obtained from other species were not assayed at this time.

  Hybridokine is also recommended for in vitro immunization procedures.

- Quality

Sterile Filtration through a capsule filter with a pore size of 0.2 mm.
Sterility testing according to USP 30 guidelines (sampling by filtration).
Mycoplasma detection in growth agar and broth.
Endotoxin quantitation as determined by the Limulus Amebocyte Lysate Gel Clot Assay; < 10 ng/mL.
Biological activity determined by cloning and fusion efficiency assays.

Advantages of Hybridokine versus Feeders

Convenient - Dilute with growth medium and add hybridoma cells !

Sterile - Contamination due to feeder cell layers is eliminated.

Low Endotoxin Levels - Some feeder cell layers or conditioned media contain high levels of endotoxin.

No Contaminating Murine Antibody - High background due to murine antibody from the murine feeder cells is eliminated.

No Refeeding - Only the hybridomas utilize the medium nutrients.

Cost Effective - The need for a large animal colony is eliminated.

Cell Line Tested - The murine cell line used for production of this product tested negative for Mycoplasma and Murine Adventitious Virus (MAP testing).

Directions for use

- Reconstitute with 12.5 ml of sterile distilled water.

***Note**:* Hybridokine is stable several days at 4°C. Once reconstituted, it is recommended that the product be aliquoted into single use volumes so that freeze-thaw cycles will not be required.

- Supplement your usual culture medium:
Recommended concentration for use: 2.5% (V/V) for cell culture, up 10% for cloning step.

***ATTENTION** :* The Hybridokine is not used in place of serum, thus the culture medium should be supplemented with FCS.

Notes for hybridoma culture during HAT selection after fusion, cloning and cryopresevation:

Fusion and selection

Perform fusion of splenocytes and myelomas according to established protocol.
Centrifuge cells to remove polyethylene glycol.
Resuspend the newly fused hydridomas in HAT selection medium supplemented with 10% hydridokine, at a density of 5.10^4 to 5.10^5 splenocytes per ml. Distribute cells into microplate culture-treated wells (FPlyte).
No refeeding is necessary; growing colonies should be visible after ten days culture. Supernatants can be assayed for antibody secretion.

Cryopreservation

Antibody positive hydridomas should be expanded for cryopreservation (and following cloning steps) using hybridoma growth medium supplemented with 5% Hybridokine.
For thawing, place each vial fo frozen cells in a 37°C water bath. As soon ice has melted, pipette cell suspension in a vial filled with pre-warmed growth medium (at least 5 volumes of defrosted vial). Mix and centrifuge. Remove supernatent and resuspend with growth medium supplemented with 5% Hybridokine.

Cloning

Use 10% Hybridokine supplementation until hybridoma grows in the logarithmic phase (ca 5.10^5 cells/ml).
Count cells (use UptiBlue reagent). Dilute cells with growth media supplemented with 10%(or 2.5%*) hydridokine and at least 3%* serum to a density of 5 cells/ml (down to 1 cell/ml for further cloning steps).
*these are recommended starting concentrations, that may be optimized depending 1/on your specific hydridoma requirements, 2/on culture step and used method (flask, roller, spinner, bioreactor). Down 2.5% may be sufficient for some hydridoma and re-culture.
Dispense 0.2ml of cell suspension to each well of cell-culture treated (FPlyte) and allows for 10-15 days culture.
Assay for antibodies the supernatant of wells containing (single) colonies.
The most interesting antibody positive hybridoma may be recultured with 5% hybridokine (i.e. expanded in 24-well plates) for a second cloning step if necessary, and/or for further antibody characterization. Other hybridoma wells can be frozen for a second round cloning or selection.

Literature

I. DLeij, L., Schwander, E. and T.H. The. Cryopreservation in hybridoma production. In : methods of hybridoma formation. Eds. Bartal, A., and Hirshaut, Y., The Humana Press, Clifton, New Jersey, **419-427**, (1987).

II. Boldicke, T., Kindt, S., Mayvald, F., Fitzlaff, G., Bocher, M., Frank, R., and J. Collins. Production of specific monoclonal antibodies against the active sites of human pancreatic secretory trypsin inhibitor by in vitro immunization with synthetic peptides. Eur. J. Biochem. 175, **259-264** (1988).

Related products and documents:

See BioSciences Innovations catalogue and e-search tool.
UptiBlue viable cell counting reagent #669412
FPlyte microplates

Technical notice NT-82643

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

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