



## Hybridoma Growth Medium With TX-HYB Hybridoma Growth Supplement

### PROTOCOL

- Description** The serum-free Hybridoma growth medium is a chemically defined culture medium supporting the serum-free growth of various hybridomas and 293 cells. This protocol can be followed when using the TX-HYB supplemented Hybridoma growth medium in combination with a 1000ml cell Bioreactor.
- Please note: our protocol differs from the protocols that are in general enclosed with cell Bioreactors.  
We observed maximum hybridoma harvests when performing the following procedure.
- Preparation** Prepare the serum free Hybridoma growth medium by dissolving 1 bottle of TX-HYB (11 ml) into 1 Liter of D-MEM F-12 (Dulbecco's Modified Eagle's Medium F-12).
- 50 ml of fresh Hybridoma growth medium for the equilibration
  - 20 ml of fresh Hybridoma growth medium for the preparation of the inoculums
  - 930 ml of fresh Hybridoma growth medium for the culture of the cells
- Material**
- 1000 ml (1 Liter) cell Bioreactor
  - Standard 25 ml serological pipettes with pipetting aid
  - Pre-culture of totally 30 to 50 x 10<sup>6</sup> viable cells
- Equilibration** Equilibrate the cell Bioreactor by putting 50 ml of prepared Hybridoma growth medium into the medium compartment and let the semi-permeable membrane equilibrated for at least 5 minutes.
- Inoculum preparation** Obtain 30 - 50 x 10<sup>6</sup> viable cells from a pre-culture in log growth phase (about 18 x T75 flasks) and suspend the cells in 20ml prepared Hybridoma growth medium.
- Inoculation** To inoculate the cell Bioreactor, loosen the front cap of the medium compartment in order to prevent air lock. Aspirate the 20 ml cell suspension into a pipette, open the cell compartment and inoculate the cell compartment by inserting the pipette into the black silicone cone.
- It is important to minimize the introduction of air bubbles into the cell compartment during seeding. In case air gets trapped within the cell compartment try to carefully remove the big bubbles by carefully drawing them back into the pipette together with fluid. Close the cell compartment by completely tighten the cap.
- After seeding, add 930 ml of equilibrated Hybridoma growth medium into the medium compartment and then completely tighten both caps. Place the cell Bioreactor into a standard CO<sub>2</sub> incubator under culture conditions appropriate for your individual cell type.

**Harvest**

In general, when using TX-HYB supplemented Hybridoma growth medium in combination with a cell Bioreactor, the harvest is only performed when the cells are dead (shrunk morphology; about 14 days after inoculation).

Discard all medium from the medium compartment.  
Loosen the medium compartment cap.

Gently harvest all liquid from the cell compartment by aspirating the content with a 25 ml serological pipette. Slowly pipette the liquid up and down several times to thoroughly mix the cell suspension.

The cell compartment will comprise about 20 ml cell suspension with the individual secreted product. Due to osmotic flux of liquid from the medium compartment to the cell compartment, the total volume might be slightly increased. The cell compartment harvest can be further purified according to the recommended purification method for your secreted product.

**Re-usage**

Wash the cell Bioreactor cell compartment with PBS. Remove the PBS and add trypsin to the cell compartment. Wait until all dead cells are removed from the cell membrane. Remove the trypsin and wash the cell compartment again with PBS.

Repeat the whole washing step 3 times (PBS → trypsin → PBS).  
When adding 1000 ml of fresh TX-HYB supplemented Hybridoma growth medium in the medium compartment, the cell Bioreactor can be placed back into the incubator until next use.

Note: only use the cell Bioreactor again when using the same hybridoma cell line, which is secreting the same product. When you start using another hybridoma cell line, another cell Bioreactor has to be used to avoid mixing-up hybridoma cells and secreted products.

**Expiry**

The prepared Hybridoma growth medium can be stored at 2°C – 8°C for at least 3 months.

**Disclaimer**

The TX-HYB Hybridoma Growth Supplement is for laboratory use only. It is not to be used as drug, for household or other uses. Although certain products may be used for investigation procedures in animals, neither the animals used in such studies or any of the resulting products may be used as food for humans or other animals.

---

Gentaur Molecular products  
Avenue de l'Armée 68/B4  
B1040 Brussel  
Belgium

Tel : +32 16 58 90 45  
Fax : +32 16 50 90 45  
info@gentaur.com  
support@gentaur.com

[www.gentaur.com](http://www.gentaur.com)  
[www.bioxys.com](http://www.bioxys.com)  
[www.genprice.com](http://www.genprice.com)  
[www.gencompare.com](http://www.gencompare.com)