Product Instruction

Hybridoma Cell Serum-free Medium

Product Type: Hyber-B100, Hyber-F100

Product Description

Hybridoma cell serum-free medium (Hyber-B100, Hyber-F100) is developed by Shanghai BioEngine Sci-Tech Co., Ltd. This medium is suitable for the effective expressions of high-density suspension culture and antibody protein of hybridoma cell.

Product Formula

The intellectual property rights of hybridoma cell serum-free medium (Hyber-B100, Hyber-F100) formula are owned by Shanghai BioEngine Sci-Tech Co., Ltd. For additional information, please contact our technical support department.

Product Ingredient

The medium contains carbohydrates, amino acids, vitamins, hydrolysate and other nutritional components.

This product does not contain components of animal origin, genetically modified plant origin or raw material with mad cow virus origin.

Product Preservation

- Store in a dark environment at 2-8℃.
- Do not recommend to use, when the product is beyond expiration date.

Use of Products

- Hyber-B100

Suspension adaption of adherent cells

For adherent hybridoma cells, this medium can be used for serum-free suspension adaption.

Direct adaption

(1) Select adherent cells with confluent degree of more than 80%, discard the culture medium,

and wash them once with aseptic PBS. After absorbing all the liquid, add 0.25% trypsin to fully infiltrate the cells, discard the excess trypsin, and digest at room temperature until the cells become round (about 1-2 minutes). Use the basal medium containing serum to terminate digestion. Blow the cells down completely.

(2) The digested cells were centrifuged at 1000 rpm for 5 minutes, the supernatant was discarded, and the cells were suspended with Hyber-B100 medium and transferred to a 125 ml shake flask with the density controlled at 0.5-0.8×10^6 cells/ml.

(3) The culture was incubated at 37℃ with 5% CO_2 on a shaker with a rotating speed of 110-130 rpm.

(4) After 48 hours of culture, if the cell density reached 1.0-3.0 × 10^6 cells/ml and the viability was higher than 90%, the cells were diluted to 0.5-0.8×10^6 cells/ml.

(5) Steps (3) - (4) were repeated until the specific cell growth rate was higher than 0.45d^{-1} for more than five consecutive passages and the cell viability was higher than 90%, then serum-free acclimation was considered successful.

Gradual adaption

(1) Select adherent cells with confluent degree of more than 80%, discard the culture medium, and wash them once with aseptic PBS. After absorbing all the liquid, add 0.25% trypsin to fully infiltrate the cells, discard the excess trypsin, and digest at room temperature until the cells become round (about 1-2 minutes). Use the basal medium containing serum to terminate digestion. Blow the cells down completely.

(2) The digested cells were suspended with the medium prepared by the original medium and Hyber-B100 in a certain proportion, and then transferred to a 125 ml shake flask with the seeding density controlled at 0.5-0.8×10^6 cells/ml.

(3) The culture was incubated at 37℃ with 5% CO_2 on a shaker with a rotating speed of 110-130 rpm.

(4) After 48 hours of culture, if the cell density reached 1.0-3.0 × 10^6 cells/ml and the viability was higher than 90%, the proportion of Hyber-B100 serum-free medium was increased as acclimation medium for passage. Otherwise, the original acclimated medium was still used for adaption.

Suggestion: The proportion of acclimated medium containing Hyber-B100 serum-free medium was 25%, 50%, 90%, 95%, 99% and 100%, respectively.

Serum-free acclimation was considered successful when complete Hyber-B100 medium was used for stable passage (cell specific growth rate was stable above 0.45d^{-1} and viability was stable above 90%).

Suspension cell adaptation

The original serum-free medium was gradually replaced by dilution and subculture method, as follows: Hyber-B100 medium was used for dilution and passage every 24 or 48 hours, and the viable cell density was controlled at 1.0-2.0×10^6 cells/ml after subculture. When the specific growth rate of cells was stable and more than 0.6 day^{-1} after more than three consecutive passages, the cells were considered to be adapted to the medium.

Cell passage and expansion

Hyber-B100 medium was used every 24 or 48 hours for dilution passage and expansion, and the viable cell seeding density was controlled at 0.5-1.0×10^6 cells/ml.

Batch culture

It is suggested to adapt hybridoma cells in this medium, and then batch culture. The cells with activity greater than 90% in the exponential growth phase were selected for batch culture, and the seeding density was 1.0-1.5×10^6 cells/ml.

- Hyber-F100

Fed-batch culture

(1) The hybridoma cells were acclimated in Hyber-B100 medium, and then cultured in stream.

(2) Select cells in exponential growth phase with activity greater than 90%.

(3) Hyber-B100 medium was used for dilution passage, and the seeding density was controlled at 1.0-1.5×10^6 cells/ml.

(4) From the first day to the sixth day of culture, 3% (v/v) Hyber-F100 flow was added to the culture medium daily.

(5) It is recommended to terminate the fed-batch process when the fed-batch culture period

exceeds 7 days or the cell activity is lower than 50%.

Suggestion: For protein expression, the effect of Hyber-B100 combined with Hyber-F100 was better than that of Hyber-B100 alone.