Serum free medium for CHO cells

with L-Alanyl-L-Glutamine, with F-68, sterile-filtered, animal-free, chemically defined, suitable for middle to high-density suspension culture of CHO cells and recombinant protein expression

Catalog number: LF-KR- ACE06101

Product Form: Clear solution at 1x

Product size: 1000 mL

Sterility: Sterile-filtered

pH: 6.80~7.60

Endotoxin (LAL test): ≤ 5 EU/mL

Shipment: With blue ice

Storage: Store at 2-8 °C for 9 months

Application: This product is a serum-free, chemically defined medium (mainly containing carbohydrates, amino acids, inorganic salts, trace elements, etc) for middle-to-high-density suspension culture of CHO and other CHO cells, as well as recombinant protein expression.

Usage instruction:

1. Cell Medium Change and Subculture

(1) Cells received at room temperature should be immediately transferred to a suitable shake flask (the volume of the culture medium should be controlled within one-fifth of the capacity of the shake flask) and placed in a shaker for shaking culture. When the cell density returns to 3-6×10^6 cells/ml, subculture or cell cryopreservation can be carried out;

(2) Cells taken out from the liquid nitrogen tank or transported with dry ice should be resuscitated and cultured according to the method described in (**3. Cell Cryopreservation and Resuscitation**) below;

(3) During subculture, it is necessary to count the cells first. After confirming the density, there is no need to centrifuge the cells, and the cell suspension can be directly mixed into the culture medium in the required proportion. The cell density after subculture should be controlled at 0.3×10^6 cells/ml, and subculture is generally required once every 4 days; or the subculture density is 0.6×10^6 cells/ml, and subculture is performed once every 3 days. This culture medium can support a maximum cell density of about 1×10^7 cells/ml, and the survival rate of cells can generally remain above 95% when reaching this density;

(4) If there are too many dead cells during shaking culture, low cell density can be inoculated for static culture, and shaking culture can be carried out after the cells return to normal viability.

2. Cell Cryopreservation and Resuscitation

3.1 Cryopreservation

(1) Prepare 7.5% dimethyl sulfoxide (DMSO) cell cryopreservation solution with cell culture medium;

(2) Culture the cells to the logarithmic growth phase (density is about 6×10^6 cells/ml), count and centrifuge to collect the cells;

(3) Resuspend the centrifuged cells with cell cryopreservation solution at a concentration of $5\text{-}20\times10^6$ cells/ml;

(4) Dispense the cell suspension into labeled cryopreservation tubes, ensuring that the tube caps are tightened to make them completely sealed;

(5) Place the cryopreservation tubes in a -80°C refrigerator for slow cooling;

(6) Transfer the cryopreservation tubes to liquid nitrogen for long-term storage the next day. This process should be completed as quickly as possible (it is recommended to finish within 2 minutes). If the temperature of the cryopreservation tube rises above -50°C during this

process, the cells may be quickly damaged.。

3.2 Resuscitation

(1) Take out the cell cryopreservation tube from the liquid nitrogen tank, dry ice or ultra-low temperature refrigerator, and immediately place it in warm water at 37°C until the ice crystals in the tube are completely melted;

(2) After thawing, thoroughly wipe the cryopreservation tube with 75% ethanol, transfer all the cell suspension in the tube to a 50ml sterile centrifuge tube with a pipette, and slowly add cell culture medium to a final volume of 20ml;

(3) Centrifuge at 1000rpm for 5 minutes, and discard the supernatant containing the cryopreservation solution;

(4) Resuspend the cells with fresh ACE CHO SFM medium to a density of 0.6-1.0×10^6 cells/ml, and inoculate them into a shake flask for shaking culture. Count the cells after 3-6 days of culture, and observe whether the cell density and survival rate increase. If the cell density reaches 3-6×10^6 cells/ml, the cells can be subcultured. Generally, the cells can return to a normal growth state after 1-2 subcultures;

(5) Cells cryopreserved with Zhuhai Kairui serum-free cell cryopreservation solution can be directly resuscitated and melted, then transferred to a shake flask with ACE CHO SFM medium added for resuspension. Count the cells after 3-6 days of culture, and observe whether the cell density and survival rate increase.

(6) If the cell density is too low after thawing, they can be first inoculated into a culture flask for static culture. When the density returns to 0.6-1.0×10^6 cells/ml, they can be placed in a shaker of the incubator for shaking culture.