Transpro CD 01 (-Gln)

High-Performance Culture Medium for Animal-Cells

Product Instruction Manual

DUONING

High-Performance Culture Medium for Animal Cells

Product Name: Transpro CD 01 (-Gln) Medium
Main Product No.: MP040; Powder packaging

Product Description

Transpro CD 01 (-Gln) medium is a universal transient medium, which can be used for subculture, high-density culture and transient transfection culture of HEK293 cells and CHO cells. The transient transfection process does not require centrifugation to exchange the medium. Transpro CD 01 (-Gln) medium is suitable for the use of 293 series cells suchasHEK293, Expi293F, 293F, 293E and CHO series cells such as ExpiCHOS and CHOS for transient transfection expression culture of antibodies, recombinant proteins and viruses during the development and manufacture process. TransproCD01(-Gln) medium is an animal-derived component free (ACF), protein free (PF), chemically defined (CD) medium, and does not contain any growth factor and hydrolysates, which ensures consistency between batches and improves the efficiency of the cell culture process. This product does not contain HT, anti-clumping agent or L-Glutamine.

Preparation Guide

Suitable for powder packaging (take 1L as example)

1) Prepare ultrapure water with a volume of about 90% (900 mL);
2) Add 22.80 g of Transpro CD 01 (-Gln) medium powder, and stir for 30 min to dissolve completely;
3) Add sodium bicarbonate 2.220 g, and stir for 5~10 min until completely dissolved;
4) Fix volume, stirring for 5 ~ 10 min;
5) Measure pH and osmotic pressure;(Adjust pH 7.00 to 7.40, Osmolality 275 to 320 mOsm/kg);
6) Sterilize with a 0.22 μm filter.

Cell Culture

1) Suggested cell inoculation density: 0.2 – 1.0 x 10^6 cells/mL.
2) Temperature: 36.5℃
3) CO_2: 8%

Cell Adaption

Most cell lines use this product without any adaption and can be directly inoculated into this medium and passaged for more than three times. For some cell lines, gradient continuous adaption may be used when using this series of medium.

Cell Cryopreservation

1) Prepare the cryopreservation solution on the ultraclean workbench: 90% Transpro CD 01 + 10% dimethyl sulfoxide (DMSO) mixture, precooling at 2~8°C(Temperature will be released when DMSO is diluted);
2) Cryopreserved cell suspension: in exponential growth stage, with a density greater than 1.5 x 10^6 cells/ml, and the viability is greater than 95%;
3) The cell suspension was centrifuged at 800 rpm for 5 min;

4) Slowly pour out the supernatant and resuspend the cells with cryopreservation solution, and the cryopreservation density is 1.0 ~ 1.5×10^7 cells/ml, transfer the cells to the sterile cryopreservation tube;
5) Place the cryopreservation tube in the cryopreservation box containing isopropyl alcohol, freeze it at - 80 ℃ overnight, and then transfer it to the liquid nitrogen tank for long-term storage. If there is no freezing box, the temperature can be reduced manually by gradient as follows:
   - Freeze at 4 ℃ for 30 min;
   - Freeze at -20 ℃ for 2-4 h;
   - Freeze at -80 ℃ overnight;
   - Transfer frozen cells to liquid nitrogen tank for long-term storage.

Cell Recovery

1) Prepare 37 ℃ warm water to thaw frozen cells;
2) Prepare a 15mL sterile centrifuge tube and add 2 ~ 5mL Transpro CD 01;
3) Take out the cryopreservation tube from the liquid nitrogen tank and rapidly thaw frozen cells in a 37℃ water bath;
4) After wiping the cryopreservation tube with 75% ethanol, open the cryopreservation tube in the aseptic ultraclean workbench, transfer the cell suspension to a 15 mL centrifuge tube and centrifuge at 800 rpm for 5 min;
5) Slowly pour out the supernatant, resuspend it with 15 ~ 20 mL preheated Transpro CD 01, and transfer it to a 125 mL shake flask;
6) Place it in a shaking incubator with 8% CO_2, 110 ~ 130 rpm, at 36.5℃ for culture;
7) After 2-3 days of culture, the cells are counted and subcultured.

Cell passage

The cells are seeded at 0.2 ~ 1.0×10^6 cells/mL, count and subculture every 2 ~ 3 days. In the first three passages, the volume remained unchanged to restore cell viability. After the cell viability recovers to normal and reaches more than 90%. The seed cells were expanded at the density of 0.2 ~ 1.0×10^6 cells/mL until reaching the required volume. The criteria for normal seed state: the viability was greater than 95%, the cell morphology was regular and round, and the growth doubling time was normal.

Transient Transfection Operation

1) The day before transfection need to seed cells at 2.0x10^6 cells/ml, the cell density can reach 4.0x10^6 cells/ml on the second day;
2) After cell counting on the second day of culture, the cell viability was more than 95%, and the viable cell density was ⩾4.0x10^6 cells /ml, can be used directly; If the cell density is lower than 4.0x10^6 cells /ml, the cells can be collected by centrifugation (800 rpm, 5 min), and the cells can be resuspended in Transpro CD01 medium at density of 4.0x10^6 cells/ml;
3) The mixture of DNA and PEI was prepared according to the optimized transient transfection process;
4) Add the mixed solution to the medium for culture;
5) After 18 hours of culture, it is recommended to supplement the supplemented medium Transpro feed 1 (the concentration is recommended to be 3-5% of the initial culture volume), or the combined supplemented medium DN feed B2 (the concentration is recommended to be 0.3-0.5% of the initial culture volume), which can further improve the density of viable cells and protein production;
6) Culture for 7 days, or when the viability is less than 60%, end the culture.

Storage and Retest Date

Transpro CD 01 (-Gln) medium powder packing: 2℃ to 8℃, Protect from light; retest date: 24months.

Manufacturer Information

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