Transpro CD 01

High-Performance Culture Medium for Animal-Cells

Product Instruction Manual

DUONING

High-Performance Culture Medium for Animal Cells

Product Name: Transpro CD 01 Medium
Main Product No.: MP004; Powder packaging

Product Description

Transpro CD 01 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 change the medium. Transpro CD 01 is suitable for the use of 293 series cells such as HEK 293, 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. Transpro CD 01 is an animal-derived component free (ACF), protein free (PF), chemically defined (CD) medium. Transpro CD 01 medium 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 and anti-clumping agent. Transpro CD 01 liquid package does not contain L-glutamine, it needs to be supplemented with 4-6 mM L-glutamine when used. Transpro CD 01 medium powder package contains 6 mM L-glutamine.

Preparation Guide

Suitable for powder packaging (take 1L as example)

1) Prepare ultrapure water with a volume of about 90% (20-30 ℃);
2) Add 0.165 g of Duoning medium additive powder, stir for 5 min;
3) Add Transpro CD 01 medium powder 23.51 g, stir for 10 min;
4) Add sodium bicarbonate 2.220 g;
5) Stir for 30 min until completely dissolved;
6) Adjust pH to 7.00~7.40;
7) Fix volume, stir for 5~10 min;
8) Sterilized with 0.22μm filter.

Cell Culture

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

Cell Adaption

Most cell lines can adapt directly to this product. They can be directly inoculated into this medium and passaged more than three times. For more cell lines, sequential cell adaption may be used when using this medium.

Cell Recovery

1) Prepare a 36.5 °C warm water to thaw cells;
2) 15 ml sterile centrifuge tube is prepared, and 2-5mL Transpro CD 01 is added;
3) Take out the cryopreservation tube from the liquid nitrogen tank and rapidly thaw (<2 minute) frozen cells in a 36.5°C water bath;

4) After wiping the cryopreservation tube with 75% ethanol, open the cryopreservation tube in the sterile operation table, transfer the cell fluid to a 15 ml centrifuge tube containing 2-5 mL of Transpro CD 01, blow and mix well, centrifuge at 800 rpm for 5 minutes;
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, 110~130rpm, 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 $\times 10^6$ cells/ml, count and subculture every 2~ 3 days. In the first three passages, the volume remained unchanged to restore cell viability. When the cell viability returned to normal and reached more than 90%, it was expanded from 0.2 ~ 1.0$\times 10^6$ cells/ml until the required seed volume and normal seed state were reached: the viability was greater than 95%, the cell morphology was regular and round, and the growth doubling time was normal.

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 1.5x10^6 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$\times 10^7$ 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

Transient Transfection Operation

1) The day before transfection need to seed cells at 2.0x10^6 viable cells/ml, the cell density can reach 4.0x10^6 viable 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 living 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 culture 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 expression;

6) Culture for 7 days, or the viability is less than 60%, and end the culture.

Storage, Validity Period or Retest Date

Shanghai production base, powder packaging: 2°C to 8°C, protect from light; validity period: 24 months.

Wuxi production base, powder packaging: 2°C to 8°C, protect from light; retest date: 24 months.

Manufacturer Information

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