产品使用说明书 Product Instruction Manual

多宁/DuoNing

动物细胞高性能培养基 High-Performance Culture Medium for Animal-Cells

V165-01

【产品名称 Product name】**Transpro CD 01 plus**

【主货号 Main Art. No.】 **M021**　　液体包装 Liquid packaging

【产品说明 Product description】

Transpro CD 01 plus 是一种通用型培养基，该产品可用于悬浮 HEK293 细胞的腺病毒的生产，同时也可用于 HEK293 细胞和 CHO 细胞的传代培养、高密度培养和瞬时转染培养。Transpro CD 01 plus 适合采用 HEK 293、Expi293F、293F、HEK 293-SF-3F6、293E 等 HEK293 系列细胞进行研发过程中腺病毒、慢病毒、腺相关病毒等的生产。同时 Transpro CD 01 plus 也适合采用 HEK 293、Expi293F、293F、293E 等 HEK293 系列细胞和 CHOK1、CHOZN 等 CHO 系列细胞进行研发过程中抗体、重组蛋白的瞬时转染表达培养。该产品是完全化学成分限定培养基、无动物来源成分、无蛋白成分、无动物或植物来源蛋白水解物。Transpro CD 01 plus 液体包装不含有 L-谷氨酰胺，使用时需额外补加 4-6mM L-谷氨酰胺。

Transpro CD 01 plus is a universal culture medium that can be used for the production of adenovirus in suspension of HEK293 cells, as well as for passaging, high-density culture, and transient transfection culture of HEK293 and CHO cells. Transpro CD 01 plus is suitable for the production of adenoviruses, lentiviruses, adeno-associated viruses, etc. during the research and development process using HEK 293 series cells such as HEK 293, Expi293F, 293F, HEK 293-SF-3F6, 293E, etc. Meanwhile, Transpro CD 01 plus is also suitable for transient transfection and expression culture of antibodies and recombinant proteins during the development process using HEK293 series cells such as HEK 293, Expi293F, 293F, 293E, and CHOK1, CHOZN, and other CHO series cells. This product is a fully chemically limited culture medium, free of animal derived ingredients, free of protein components, and free of animal or plant derived protein hydrolysates. Transpro CD 01 plus liquid packaging does not contain L-glutamine, and an additional 4-6 mM L-glutamine needs to be added during use.

【细胞培养 Cell culture】

① 建议细胞接种密度 Suggested cell inoculation density：0.2~1.0×10^6 cells/mL.

② 温度 Temperature：37℃

③ CO_2:8%

④ 使用时需要额外添加 L-谷氨酰胺 4-6mM

It needs to be supplemented with 4-6mM L-glutamine when used.

【细胞驯化 Cell domestication】

多数细胞株使用本产品是不需要任何驯化，直接接种到本培养基，传代三次以上即可。对有些细胞株，使用本系列培养基时可能要采用梯度连续驯化。

Most cells lines can adapt directly into this product. They can be directly inoculated into this medium and passed more than three times. For some cell lines, sequential cell adaptation may be used when using this medium.

【细胞冻存 Cell cryopreservation】

① 在超净工作台上准备冻存液：90% Transpro CD 01 plus+ 10% 二甲基亚砜（DMSO）混合液，2~8℃预冷（DMSO 稀释时会释放热量）；

Prepare frozen solution on the super clean workbench : 90% Transpro CD 01 plus +10% dimethyl sulfoxide (DMSO) mixed solution, precooling at 2~8℃ (heat will be released when DMSO is diluted);

② 冻存细胞液：处于对数生长期，密度大于 1.5×10^6cells/mL，活率大于 95%；

Frozen cell fluid: cells are in the exponential growth period, with a density greater than 1.5×10^6cells/mL, and the viability is greater than 95%.

③ 细胞液 800rpm 离心 5 min；

Cell fluid was centrifuged at 800rpm for 5 min；

④ 缓慢倒出上清液，使用冻存液重新悬浮细胞，冻存密度 $1.0\sim1.5\times10^7$cells/mL，将细胞转移至无菌冻存管中；

Slowly pour out the supernatant, resuspend the cells with cryopreservation solution, the cryopreservation density is $1.0\sim1.5\times10^7$cells/mL, and transfer the cells to a sterile cryopreservation tube;

⑤ 将冻存管置于含异丙醇的冻存盒中，-80℃冻存过夜，再转移至液氮罐中长期贮存。如果没有冻存盒，可手动梯度降温，步骤如下：

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:

- 4℃冻存 30min；
- freeze at 4℃ for 30min；
- -20℃冻存 2~4 小时；
- freeze at -20℃ for 2~4h；

- -80℃冻存过夜；
- freeze at - 80℃ overnight；
- 转移至液氮罐中长期贮存。
- transfer frozen cells to liquid nitrogen tank for long-term storage.

【细胞复苏 Cell resuscitation】

① 准备 37℃ 温水，用于解冻细胞；

Prepare a 37 ℃ warm water to thaw frozen cells;

② 准备 15 ml 无菌离心管，加入 2~5mL 的 Transpro CD 01 plus；

Prepare 15 ml sterile centrifuge tube and add 2~5mL Transpro CD 01 plus;

③ 从液氮罐中取出冻存管，迅速在 37℃ 温水中将细胞融化；

Take out the frozen tube from the liquid nitrogen tank and quickly thaw frozen cells in 37℃ warm water;

④ 用 75%的乙醇擦拭冻存管后，在无菌操作台中打开冻存管，将细胞液转移至含 2~5 mL 的 Transpro CD 01 plus 的 15 ml 离心管中，吹打混匀，800rpm 离心 5 min；

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 plus, blow and mix well, centrifuge at 800 rpm for 5 minutes;

⑤ 缓慢倒出上清液，使用 15~20 ml 预热 Transpro CD 01 plus 重新悬浮，转移至 125 ml 摇瓶中；

Slowly pour out the supernatant, resuspend with 15~20 ml preheated Transpro CD 01 plus, and transfer to a 125 ml shake flask;

⑥ 放置于 37℃，8% CO_2，80%湿度，110~130rpm 的摇床中培养；

Place it in a shaking incubator with 8% CO_2，80% humidity, 110 ~ 130rpm，at 37℃ for culture;

⑦ 培养 2~3 天后，对细胞进行计数传代。

After 2~3 days of culture, the cells were counted and subcultured.

【细胞传代 Cell passage】

种子细胞按照 0.2~1.0×10^6 cells/mL 的密度进行传代，每隔 2~3 天计数，传代。前 3 次传代，体积不变，以恢复细胞活力。待细胞活力恢复正常，达 90%以上后，以 0.2~1.0×10^6 cells/mL 的密度进行扩增，直至达到所需种子体积，种子状态正常的标准：活力大于 95%，细胞形态规则圆整，生长倍增时间正常。

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.

【细胞瞬转 Transient transfection operation】

①转染前一天按照 2.0×10^6 cells/mL 密度接种，培养第二天细胞密度可至 4.0×10^6 cells/mL 左右；

The day before transfection need to seed cells at 2.0×106 viable cells/ml，the cell density can reach 4.0×10^6 viable cells/ml on the second day ;

②培养第二天细胞计数后，细胞活率＞95%，活细胞密度≥4.0×10^6 cells/mL，可直接使用；若细胞密度低于 4.0×10^6 cells/mL，可通过离心（800rpm，5 min）收集细胞，将细胞以 4.0×10^6 cells/mL 密度重悬于 Transpro CD 01 plus 培养基中；

After cell counting on the second day of culture, the cell viability was more than 95%, and the living cell density was ≥ 4.0×10^6 cells / ml, can be used directly; If the cell density is lower than 4.0×10^6 cells / ml, the cells can be collected by centrifugation (800 rpm, 5 min), and the cells can be separated at 4.0×10^6 cells / ml density was resuspended in Transpro CD 01 plus medium;

③ 按照优化后的瞬转工艺，制备 DNA 和 PEI 混合液；

The mixture of DNA and PEI was prepared according to the optimized transient process;

④将混合液加入到培养液中，进行培养；

Add the mixed solution to the culture medium for culture;

⑤培养 18h 后，建议补加补料培养基 Transpro feed 1（浓度建议为初始培养体积 3-5%），或者组合补加补料培养基 DN feed B2（浓度建议为初始培养体积 0.3-0.5%），可进一步提高活细胞密度和蛋白表达量。

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 living cells and protein expression.

⑥培养至 7 天，或者活力低于 60%，结束培养。

Culture until 7 days, or the vitality is less than 60%, and end the culture.

【储存和有效期 Storage and validity period】

Transpro CD 01 plus 液体包装：2~8℃避光储存，有效期为 12 个月。

Transpro CD 01 plus liquid packaging: 2℃ to 8℃, protect from light; validity period: 12 months.

【生产企业信息 Manufacturer information】

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