产品使用说明书 Product Use Instructions

多宁/DuoNing

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

V191-00

【产品名称 Product name】Transpro CD 01 (-Gln)培养基　Transpro CD 01 (-Gln) medium

【主货号 Main Art. No.】MP040　　粉末包装 Powder packaging

【产品说明 Product description】

Transpro CD 01 (-Gln)培养基是一种通用型瞬转培养基，该产品可同时用于 HEK293 细胞和 CHO 细胞的传代培养、高密度培养和瞬时转染培养，瞬时转染过程中不需要离心换液。Transpro CD 01 (-Gln)培养基适合采用 HEK 293、Expi293F、293F、293E 等 HEK293 系列细胞和 expiCHOS、CHOS 等 CHO 系列细胞进行研发过程中抗体、重组蛋白和病毒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、无生长因子。本产品不包含 HT，抗结团剂和 L-谷氨酰胺。

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 change the medium. Transpro CD 01 (-Gln) medium 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 (-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.

【配制指南 Preparation guide】

适用于粉末包装（以 1L 为例）

Suitable for powder package (taking 1L as an example)

1.准备配液体积 90%左右的超纯水(900 mL)；

1.Prepare ultrapure water with a volume of about 90% (900 mL);

2.加入 Transpro CD 01 (-Gln)培养基粉末 22.80g，搅拌 30min 溶解完全；

2.Add 22.80g of Transpro CD 01 (-Gln) medium powder, and stir for 30min to dissolve completely;

3.加入碳酸氢钠 2.220g，搅拌 5~10min 至完全溶解；

3.Add sodium bicarbonate 2.220g, and stir for 5~10min until completely dissolved;

4.定容，搅拌 5~10 min；

4.Constant volume, stirring for 5 ~ 10 min;

5.检测 pH 和渗透压；（调节 pH 范围 7.00-7.40，渗透压范围 275-320mOsm/kg）

5. Detecting pH and osmotic pressure; (Adjust pH7.00 to 7.40, Osmolality 275 to 320 mOsm/kg)

6.用 0.22μm 过滤器除菌过滤。

6. Sterilize and filter with a 0.22μm filter.

【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：36.5℃

③ CO_2：8%

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

【细胞冻存 Cell cryopreservation】

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

Prepare the cryopreservation solution on the super clean workbench: 90% Transpro CD 01 + 10% dimethyl sulfoxide (DMSO) mixture, 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%L；

Cryopreserved cell fluid: in logarithmic growth stage, with a density greater than 1.5×10^6cells / mL, and the activity rate is greater than 95%;

③ 细胞液 800rpm 离心 5 min；

The cell fluid was centrifuged at 800 rpm 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 1.0~1.5×10^7cells/mL，将细胞转移至无菌冻存管中；

Slowly pour out the supernatant and resuspend the cells with cryopreservation solution, and the cryopreservation density is 1.0 ~ 1.5×10^7cells / mL, transfer the cells to the 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，用于融化细胞；

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；

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

③从液氮罐中取出冻存管，迅速在 37℃水浴锅中将细胞融化；

Take out the cryopreservation tube from the liquid nitrogen tank and rapidly thaw frozen cells in a 37℃ water bath;

④用 75%的乙醇擦拭冻存管后，在无菌操作台中打开冻存管，将细胞悬液转移至 15 ml 离心管中，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 suspension to a 15 mL centrifuge tube and centrifuge at 800 rpm for 5 min;

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

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放置于 36.5℃，8% CO_2，110~130rpm 的摇床中培养；

Place it in a shaking incubator with 8% CO_2, 110 ~ 130rpm, at 36.5℃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 are counted and subcultured.

【细胞传代 Cell passage】

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 2.0×10^6 cells/mL，the cell density can reach 4.0×10^6 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 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 resuspended at 4.0×10^6 cells/mL density in Transpro CD 01;

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 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 production.

⑥培养至 7 天，或者活力低于 60%，结束培养。

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

【储存和复验期 Storage and retest date】

Transpro CD 01 (-Gln)培养基干粉包装：2~8℃ 避光储存，复验期为 24 个月。

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

【生产企业信息 Manufacturer information】

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