产品使用说明书 Product Instruction Manual

多宁/DuoNing

高性能瞬转培养基 High-Performance Medium for Transient Transfection

V156-02

【产品名称 Product name】Transpro CD 01 培养基 Transpro CD 01 medium

【主货号 Main Art. No.】MP004

粉末包装 Powder packaging

【产品说明 Product description】

Transpro CD 01 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 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 和抗结团剂，Transpro CD 01 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 培养基粉末包装含有 6mM L-谷氨酰胺。

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 4-6mM L-glutamine when used. Transpro CD 01 medium powder package contains 6mM L-glutamine.

【配制指南 Preparation guide】

适用于粉末包装（以 1L 为例）Powder packaging (Case 1L)

1. 准备配液体积 90%左右的超纯水（20~30℃）；

   Prepare ultrapure water with a volume of about 90% (20~30℃);

2. 加入多宁培养基添加剂粉末 0.165g，搅拌 5min；

   Add 0.165g of Duoning medium additive powder, stir for 5min;

3. 加入 Transpro CD 01 培养基粉末 23.51g，搅拌 10min；

Add Transpro CD 01 medium powder 23.51g, stir for 10min；

4.加入碳酸氢钠 2.220g；

Add sodium bicarbonate 2.220g；

5. 搅拌 30min，至完全溶解；

Stir for 30min until completely dissolved；

6.调节 pH 至 7.00~7.40；

Adjust pH to 7.00~7.40；

7.定容，搅拌 5~10 min；

Constant volume, stirring for 5~10 min；

8.用 0.22μm 过滤器除菌过滤。

Sterilized with 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_2：6~8%.

【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 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.

【细胞复苏 Cell resuscitation】

①准备 36.5℃温水，用于解冻细胞；

Prepare a 36.5 ℃ warm water to thaw cells ;

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

15 ml sterile centrifuge tube is prepared, and 2~5mL Transpro CD 01 is added;

③ 从液氮罐中取出冻存管，迅速在 36.5℃ 水浴锅中将细胞融化；

Take out the cryopreservation tube from the liquid nitrogen tank and rapidly thaw (<2 minute) frozen cells in a 36.5℃ 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，将细胞液转移至含 2~5 mL 的 Transpro CD 01 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, 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 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. When the cell viability returned to normal and reached more than 90%, it was increased by 0.2 ~ 1.0×10^6 cells/ml was amplified until the required seed volume and normal seed state were reached: the vitality was greater than 95%, the cell morphology was regular and round, and the growth doubling time was normal.

【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℃(Temperature will be released when DMSO is diluted);

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

Cryopreserved cell fluid: Seed cells were 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 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 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 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、有效期或复验期 Storage condition, validity period or retest date】

上海生产基地，干粉包装：2~8℃避光储存，有效期为 24 个月。

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

无锡生产基地，干粉包装：2~8℃避光储存，复验期为 24 个月。

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

【生产企业信息 Manufacturer information】

名称 Name：上海多宁生物科技股份有限公司 Shanghai Duoning Biotechnology Co., Ltd.

地址 Address：上海市松江区民强路 1525 弄 30 幢 Building 30, Lane 1525, Minqiang Road, Songjiang District, Shanghai

电话 Tel：021-64340155　　网址 Website：www.duoningbio.com

名称 Name: 无锡多宁生物科技有限公司　Wuxi Duoning Biotechnology co.,Ltd

地址 Address: 无锡新加坡工业园新集路 2-1、2-2 号厂房　No.2-1, No.2-2, Xinji Road,Singapore Industrial Park, Wuxi

电话 Tel：0510-85956600　　网址 Website：www.duoningbio.com