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## 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

V148-01

【产品名称 Product name】 Transpro CD 01

【货号 Art. No.】 MS002-500ml、MS002-1L

液体包装 Liquid packaging

### 【产品说明 Product description】

Transpro CD 01 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。Transpro CD 01 液体包装不含有 L-谷氨酰胺、HT 和抗结团剂。

Transpro CD 01 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. Transpro CD 01 liquid package does not contain L-glutamine, HT and anti-clumping agent.

### 【细胞培养 Cell culture】


- ① 建议细胞接种密度 Suggested cell inoculation density:  $0.2\sim 1.0\times 10^6$  cells/mL
- ② 温度 Temperature: 37°C
- ③ CO<sub>2</sub>: 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

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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+10%二甲基亚砷（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°C(Temperature will be released when DMSO is diluted);

- ② 冻存细胞液：处于对数生长期，密度大于  $1.5 \times 10^6$  cells/mL，活率大于 95%，一般建议冻存密度为  $1.0 \sim 1.5 \times 10^7$  cells/mL；

Cryopreserved cell fluid: in logarithmic growth stage, with a density greater than  $1.5 \times 10^6$  cells / ml, and the activity rate is greater than 95%. Generally, the recommended freezing density is  $1.0 \sim 1.5 \times 10^7$  cells/mL;

- 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  $1.0 \sim 1.5 \times 10^7$  cells/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  $1.0 \sim 1.5 \times 10^7$  cells / 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 °C 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°C for 30min；
- -20℃冻存 2~4 小时；
- freeze at -20°C for 2~4h；
- -80℃冻存过夜；
- freeze at - 80°C 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°C水浴锅，用于融化细胞；

Prepare a 37 °C water bath to thaw frozen 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 37°C 水浴锅中将细胞融化；

Take out the cryopreservation tube from the liquid nitrogen tank and rapidly thaw (<1 minute) frozen cells in a 37°C 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 CD01 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 37°C，8% CO<sub>2</sub>，110~130rpm 的摇床中培养；

Place it in a shaking incubator with 8% CO<sub>2</sub>，110 ~ 130rpm，at 37°C 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<sup>6</sup> 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<sup>6</sup> 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<sup>6</sup> 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<sup>6</sup> 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.

### 【细胞瞬转 Transient transfection operation】

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

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

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②培养第二天细胞计数后，细胞活率>95%，活细胞密度 $\geq 4.0 \times 10^6$  cells/mL，可直接使用；若细胞密度低于 $4.0 \times 10^6$  cells/mL，可通过离心(800rpm, 5 min)收集细胞，将细胞以 $4.0 \times 10^6$  cells/mL 密度重悬于 Transpro CD01 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  $\geq 4.0 \times 10^6$  cells / ml, can be used directly; If the cell density is lower than  $4.0 \times 10^6$  cells / ml, the cells can be collected by centrifugation (800 rpm, 5 min), and the cells can be separated at  $4.0 \times 10^6$  cells / ml density was resuspended in transpro CD01 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】

液体包装：2~8°C 避光储存，有效期为 12 个月

Liquid packaging: 2°C to 8°C, Protect from light; Shelf life: 12 months.

#### 【生产企业信息 Manufacturer information】

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