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

V164-01

【产品名称 Product name】多宁培养基添加剂 Duoning medium additive

【主货号 Main Art. No.】Q009　　粉末包装 Powder packaging

【产品说明 Product description】

多宁培养基添加剂是与干粉 Transpro CD 01 培养基搭配，一同配制液体 Transpro CD 01 的产品。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。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。

The Duoning medium additive is a product prepared with dry powder Transpro CD 01 medium to prepare liquid Transpro CD 01. Transpro CD 01 is a universal transient medium that can be used for both HEK293 cells and CHO cells for passaging, high-density culture and transient transfection culture without the need for centrifugal exchange during transient transfection. Transpro CD 01 is suitable for transient transfection and expression culture of antibodies, recombinant proteins, and viruses during the research and development process using HEK293 series cells such as HEK 293, Expi293F, 293F, 293E, and CHO series cells such as ExpiCHOS and CHOS. This product is a fully chemically defined medium, no animal-derived components, no protein components, no animal or plant-derived protein hydrolysates, and no growth factors. This product does not contain HT or anti-clumping agents. Transpro CD 01 liquid package does not contain L-glutamine and requires additional supplementation of 4-6 mM L-glutamine for use. Transpro CD 01 medium powder package contains 6 mM L-glutamine.

【配制指南 Preparation guide】

以多宁培养基添加剂搭配干粉 Transpro CD 01 培养基，配制液体 Transpro CD 01 为例（配液体积 1L）

Taking Duoning medium additive combined with dry powder Transpro CD 01 medium to prepare Transpro CD 01 liquid medium as an example (1L for example)

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 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, 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 frozen solution on the super clean workbench : 90% Transpro CD 01 +10% dimethyl sulfoxide (DMSO) mixed solution, precooling at 2~8℃ (heat 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%；

Frozen cell fluid: seed cells were in the exponential growth period, the density is 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 36.5℃温水，用于解冻细胞；

Prepare 36.5°C 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 15 ml sterile centrifuge tube and add 2~5mL Transpro CD 01；

③从液氮罐中取出冻存管，迅速在 36.5℃温水中将细胞解冻；

Take out the frozen tube from the liquid nitrogen tank and quickly thaw frozen cells in 36.5°C 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 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 培养基重新悬浮，转移至 125 ml 摇瓶中；

Slowly pour out the supernatant, resuspend with 15~20 ml preheated Transpro CD 01, and transfer 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 were 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.

【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】

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