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

V185-00

【产品名称 Product name】**Media C-05 培养基　Media C-05 medium**

【主货号 Main Art. No.】MP038　　粉末包装 Powder packaging

【产品说明 Product description】

Media C-05 培养基是一种无动物来源成分、无蛋白成分、化学成分限定的基础培养基，适合采用中国仓鼠卵巢细胞（CHO）进行治疗性蛋白产品研发和生产过程中的分批培养、补料分批培养和灌流培养。Media C-05 培养基不含有 L-谷氨酰胺。适合 CHOK1、CHOK1SV、CHOS、DG44 等不同细胞株的培养。

Media C-05 medium is a chemical defined basal medium with no animal origin components, no protein, which is suitable for batch culture, fed-batch culture and perfusion culture in the development and production of therapeutic protein products by Chinese hamster ovary (CHO). Media C -05 medium does not contain L- glutamine. It is suitable for the culture of different cell lines, such as CHOK1, CHOK1SV, CHOS, DG44.

【配制指南 Preparation guide】

Media C-05 培养基粉末包装（以 1L 为例）

Media C-05 medium powder packaging (taking 1L as an example)

1. 准备配液体积 90%左右的超纯水(20 ~ 30℃);

   Prepare 90% volume ultrapure water (20 ~ 30℃);

2. 加入 Media C-05 培养基粉末 22.29 g，搅拌至粉末完全分散，并继续搅拌 10 min；

   Add 22.29 g of Media C-05 medium powder, stir until the powder is completely dispersed, and continue stirring for 10 minutes;

3. 加入 4.4mL 6M NaOH，继续搅拌 30min，此时溶液澄清；

   Add 4.4mL of 6M NaOH and continue stirring for 30 minutes until the solution is clear;

4. 加入 1.8g 碳酸氢钠，搅拌 10min；

   Add 1.8g sodium bicarbonate and stir for 10 minutes;

5. 调节 pH 至 7.00~7.40；

   Adjust the pH to 7.00 ~ 7.40;

6. 定容，搅拌 5~10 min，检测渗透压（渗透压范围在 275~325 mOsm/kg）；

Constant volume, stirring for 5 ~ 10 min, and measuring the Osmotic pressure of the solution (Osmotic pressure range is 275~325 mOsm/kg);

7. 用 0.22μm 过滤器除菌过滤, 2~8℃避光保存。

Sterilize and filter with a 0.22μm filter, and store at 2-8 ℃ in dark.

【细胞培养 Cell culture】

① 建议细胞接种密度 Suggested cell inoculation density：0.3~1.0×10^6 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驯化，具体步骤如下：

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, and the specific steps are as follows:

① 直接驯化 Direct domestication

大部分细胞株可以直接驯化至 Media C-05 中。

Most cell lines can be directly domesticated to Media C-05.

细胞接种密度 Cell inoculation density：3.0~8.0×10^5 cells/mL

至少传代 2~3 代，倍增时间正常稳定，细胞活率＞90%，表示细胞株驯化完成。

After at least 2~3 generations, the doubling time is normal and stable, and the cell viability is more than 90%, indicating that the cell strain has been domesticated.

② 连续驯化 Continuous domestication

- 细胞株在原培养基培养至指数生长期中期，细胞活率＞90%时，接种到 50%：50 %（Media C-05：原培养基）体积比配制的混合培养基中，接种密度在 3~5×10^5 cells/mL，在 36.5℃和 6% CO_2 培养。细胞培养 3~4 天达到 1×10^6 cells/mL 以上，传代；

  The cell strain was cultured in the original medium to the middle of exponential growth period, and when the cell viability was more than 90%, it was inoculated into the mixed medium with the volume ratio of 50%: 50% (Media C-05: original medium), and the inoculation density was 3~5×10^5 cells/mL, and it was cultured at 36.5℃ and 6% CO_2. The cells were cultured for 3~4 days to reach more than 1×10^6 cells/mL, and then subcultured.

- 将细胞接种到 75%：25% （Media C-05：原培养基）体积比配制的混合培养基中，接种密度在 3~5×10^5 cells/mL，在 36.5℃和 6% CO_2 培养。细胞培养 3~4 天达到 1×10^6 cells/mL 以上，传代；

  Cells were inoculated into a mixed medium with the volume ratio of 75%: 25% (Media C-05: original medium), and the inoculation density was 3~5×10^5 cells/mL, and cultured at 36.5℃ and 6% CO_2. The cells were cultured for 3~4 days to reach more than 1×10^6 cells/mL, and then subcultured.

- 将该细胞接种到 100% Media C-05 中，接种密度在 3~5×10^5 cells/mL，在 36.5℃和 6%CO_2 培养。细胞培养 3~4 天达到 1×10^6 cells/mL 以上，传代；

  The cells were inoculated into 100% Media C-05 with the inoculation density of 3~5×10^5 cells/mL, and cultured at 36.5℃ and 6%CO_2. The cells were cultured for 3~4 days to reach more than 1×10^6 cells/mL, and then subcultured.

- 在 100% Media C-05 中，至少传代 2~3 代，倍增时间正常稳定，细胞活率＞90%，表示细胞株驯化完成；

  In 100% Media C-05, at least 2~3 generations, the doubling time is normal and stable, and the cell viability is more than 90%, indicating that the cell strain has been domesticated.

- 采用本驯化程序时，若细胞还是生长很慢或活率很低，可考虑从 10：90（Media C-05：原培养基）体积比配制的混合培养基起，缓慢增加 Media C-05 的比例到 25：75，50：50，75：25，100：0；或者过程中离心收集细胞，重新进行传代。

  When adopting this domestication procedure, if the cells still grow slowly or have low activity, we can consider slowly increasing the ratio of Media C-05 to 25: 75, 50: 50, 75: 25, 100: 0 from the mixed medium prepared with a volume ratio of 10: 90 (Media C-05: original medium). Or the cells are collected by centrifugation during the process and subcultured again.

【细胞冻存 Cell cryopreservation】

① 在超净工作台上准备冻存液：90% Media C-05 + 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% Media C-05 +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 1.0~1.5×10^7cells/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 1.0~1.5×10^7cells/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 a 36.5 ℃ warm water to thaw frozen cells;

② 准备 15 ml 无菌离心管，加入 2~5mL 的 Media C-05；

Prepare 15 ml sterile centrifuge tube and add 2~5mL Media C-05;

③ 从液氮罐中取出冻存管，迅速在 36.5℃ 温水中将细胞融化；

Take out the frozen tube from the liquid nitrogen tank and quickly thaw frozen cells in 36.5℃ 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 Media C-05 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 Media C-05, blow and mix well,

centrifuge at 800 rpm for 5 minutes;

⑤ 缓慢倒出上清液，使用 20~30 ml 预热 Media C-05 重新悬浮，转移至 125 ml 摇瓶中；

Slowly pour out the supernatant, resuspend with 20~30 ml preheated Media C-05, and transfer to a 125 ml shake flask;

⑥ 放置于 36.5°C，6~8% CO_2，80%湿度，110~130rpm 的摇床中培养；

Place it in a shaking incubator with 6~8% CO_2，80% humidity, 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】

种子细胞按照 5E5 ~6E5 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 5E5 ~6E5 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 5E5 ~6E5, 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 5E5 ~6E5 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.

【储存和复验期 Storage and retest date】

无锡生产基地，Media C-05 培养基干粉包装：2~8℃避光储存，复验期为 24 个月。

Wuxi production base, Media C-05 medium powder packaging: 2℃ to 8℃, protect from light; retest date: 24 months.

【生产企业信息 Manufacturer information】

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