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

V194-01

【产品名称 Product name】Media C-01 培养基 Media C-01 medium

【主货号 Main Art. No.】MP011 粉末包装 Powder packaging

【产品说明 Product description】

Media C-01 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-01 培养基不含有 L-谷氨酰胺。适合 CHOK1、CHOK1SV、CHOS 和 DG44 等不同细胞株的培养。

Media C-01 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-01 medium does not contain L- glutamine. It is suitable for the culture of different cell lines such as CHOK1, CHOK1SV, CHOS and DG44.

【配制指南 Preparation guide】

适用于粉末包装（以 1L 为例）Suitable for 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-01 培养基粉末 23.03g，搅拌 20 min，至粉末分散开来；
   Add 23.03g of Media C-01 medium powder and stir for 20 min until the powder is dispersed;
3. 加入 3.27 mL 6M NaOH 溶液，搅拌 20min，此时溶液澄清；
   Add 3.27 mL 6M NaOH solution and stir for 20min, then the solution is clarified;
4. 加入碳酸氢钠 2.22g，搅拌 5~10min，至溶解完全；
   Add 2.22g sodium bicarbonate and stir for 5~10min until it is completely dissolved;
5. 调节 pH 至 7.00~7.40（非必需步骤）；
   Adjust the pH to 7.00 ~ 7.40 (Optional step);
6. 定容，搅拌 5~10 min，检测渗透压（渗透压范围在 280~320 mOsm/kg）；
   Constant volume, stirring for 5 ~ 10 min, and measuring the Osmotic pressure of the solution (Osmotic pressure range is 280~320 mOsm/kg);

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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，使用本培养基时可能要采用梯度连续驯化，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, gradient continuous domestication may be used when using this medium. 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-01 培养基。

Most cell lines can be directly domesticated in Media C-01 medium.

细胞接种密度：3.0~8.0×10^5 cells/ml

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。

At least 2 to 3 passages, 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-01：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 until 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-01: 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.

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- 将细胞接种到 75%：25% （Media C-01：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 the mixed medium with the volume ratio of 75%: 25% (Media C-01: 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-01 培养基，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-01 medium 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-01 培养基，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-01 medium, 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 is domesticated;
- 采用本驯化程序时，若细胞还是生长很慢或活度很低，可考虑从 10：90（Media C-01：原培养基）体积比配制的混合培养基起，缓慢增加 Media C-01 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 proportion of Media C-01 to 25: 75, 50: 50, 75: 25, and 100: 0 from the mixed medium with the volume ratio of 10: 90 (Media C-01: 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-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% Media C-01 + 10% dimethyl sulfoxide (DMSO) mixture, precooling at 2~ 8℃(heat will be released when DMSO is diluted);

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

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

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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°C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°C冻存 30min；
- freeze at 4°C for 30min；
- -20°C冻存 2~4 小时；
- freeze at -20°C for 2~4h；
- -80°C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 36.5℃温水，用于解冻细胞；

Prepare a 36.5 °C warm water to thaw frozen cells;

②准备 15 mL 无菌离心管，加入 2~5 mL 的 Media C-01；

Prepare a 15mL sterile centrifuge tube and add 2 ~ 5mL Media C-01;

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 cryopreservation tube from the liquid nitrogen tank and rapidly 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 Media C-01 的 15 ml 离心管中，吹打混匀，800rpm 离心 5 min；

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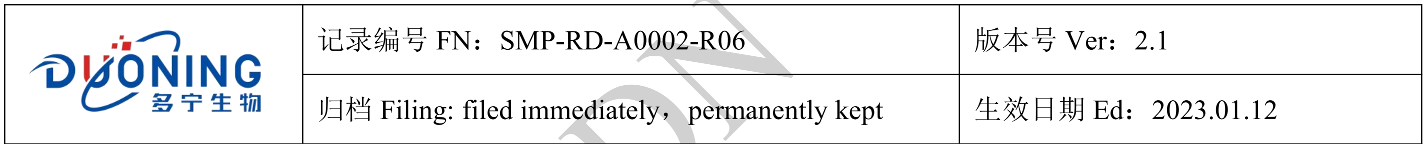

After wiping the cryopreservation tube with 75% ethanol, open the cryopreservation tube in the sterile operation table, transfer the cell fluid to a 15 ml centrifuge tube containing 2-5 mL of Media C-01, blow and mix well, centrifuge at 800 rpm for 5 minutes;

⑤缓慢倒出上清液，使用 20~30 ml 预热 Media C-01 重新悬浮，转移至 125 ml 摇瓶中；

Slowly pour out the supernatant, resuspend it with 20 ~ 30 mL preheated Media C-01, and transfer it to a 125 mL shake flask;

⑥放置于 36.5℃，6~8% CO_2，110~130rpm 的摇床中培养；

Place it in a shaking incubator with 6~8% CO_2，110 ~ 130rpm，at 36.5°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 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】

Media C-01 培养基干粉包装：2~8℃避光储存，复验期为 24 个月。

Media C-01 medium, powder packaging: 2°C to 8°C, protect from light; retest date: 24 months.

【生产企业信息 Manufacturer information】

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