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

V138-01

【产品名称 Product name】S4 CHO 干粉培养基 A 组分 S4 CHO dry powder medium - component A、S4 CHO 干粉培养基 B 组分 S4 CHO dry powder medium - component B、 S4 CHO 干粉培养基 C 组分 S4 CHO dry powder medium - component C

【主货号 Main Art. No.】MP022-A、MP022-B、MP022-C 粉末包装 Powder packaging

【产品说明 Product description】

S4 CHO 培养基是一种无动物来源成分、无血清、无蛋白成分的基础培养基，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。S4 CHO 培养基不含次黄嘌呤、胸腺嘧啶、L-谷氨酰胺，含有少量酵母和大豆来源水解物。适合采用 GS 和 DHFR 筛选系统的 CHO-K1 细胞株的培养。

S4 CHO medium is a chemical defined basal medium with no animal-derived ingredients, and no protein ingredients, 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). S4 CHO medium is free of hypoxanthine, thymine, L-glutamine and contains small amounts of yeast and soy-derived hydrolysates. Suitable for the culture of CHO-K1 cell lines using GS and DHFR screening systems.

【使用指南 User guide】

S4 CHO 培养基是为提高 CHO 细胞的生长和生产性能专门设计的培养基。

S4 CHO medium is specially designed to improve the growth and production performance of Chinese hamster ovary cell.

培养 GS 筛选系统工程细胞根据需要添加次黄嘌呤和胸腺嘧啶；

When cultivating GS screening system engineering cells, hypoxanthine and thymine were added as needed;

培养 DHFR 筛选系统工程细胞根据需要添加 2~8mM L-谷氨酰胺。

When cultivating DHFR screening system engineering cells, add 2-8mM L-glutamine as needed.

若添加胰岛素，建议添加浓度为 0.5-10mg/L。

If insulin is added, it is recommended to add a concentration of 0.5-10mg/L.

【配制指南 Preparation guide】

适用于粉末包装（以 20L 为例）Suitable for powder package (20L as an example)

1. 本培养基为低糖培养基，建议额外加入 6g/L 的葡萄糖。

   This medium is low sugar medium, it is recommended to add 6g/L glucose.

2. 准备 120g 葡萄糖，20ml 的 36.5 %浓盐酸，500mL 0.4M NaOH，42g 碳酸氢钠。

   Prepare 120g of glucose, 20ml of 36.5% concentrated hydrochloric acid, 500mL of 0.4M NaOH and 42g of sodium bicarbonate.

3. 将 42g 碳酸氢钠用 1L 纯化水溶解。

   Dissolve 42g of sodium bicarbonate in 1L of purified water.

4. 准备一个清洗干净的 20L 的玻璃烧杯或者类似容器，加入 10L 温度约为 70℃的纯化水。

   Prepare a cleaned 20L glass beaker or similar container and add 10L of purified water at a temperature of about 70℃.

5. 取配制 20L 量的 S4 CHO 干粉培养基 A 组分(19.82g/L)，边搅拌边加入到水中，搅拌 10~15 分钟。待 A 粉完全溶解后，加入 1L 含 42g 碳酸氢钠溶液（碳酸氢钠混合前先单独溶解），然后加入 120g 葡萄糖（对应 6g/L 的葡萄糖，可以按照使用要求调整），继续搅拌 10 分钟。

   Take the preparation of 20L S4 CHO dry powder medium - component A (19.82g/L), add it to the water while stirring for 10~15 minutes. When the A powder is completely dissolved, add 1L of solution containing 42g of sodium bicarbonate (dissolve sodium bicarbonate separately before mixing), then add 120g of glucose (corresponding to 6g/L of glucose, which can be adjusted according to the requirements of use) and continue stirring for 10 minutes.

6. 取配制 20L 量的 S4 CHO 干粉培养基 B 组分（1.48g/L），加入 500mL 0.4M NaOH，搅拌 10~15 分钟，待 B 粉完全溶解后，继续搅拌 10 分钟。

   Take the preparation of 20L S4 CHO dry powder medium - component B (1.48g/L) , add 500mL of 0.4M NaOH, stir for 10~15 minutes, and continue to stir for 10 minutes after the B powder is completely dissolved.

7. 待配制 A 粉的溶液降至室温后，加入配制 20L 量的 S4 CHO 干粉培养基 C 组分（25mL /L）和完全溶解的 B 粉溶液，搅拌 5~10 分钟；加入约 20ml 的 36.5 %浓盐酸调节 pH 到 6.9-7.1，定容到 20L。

   After the temperature of the solution A dropped to room temperature, add prepared 20L of S4 CHO dry powder medium - component C (25mL / L) and completely dissolved B powder solution, stir for 5~10 minutes; add about 20ml of 36.5 % concentrated hydrochloric acid to adjust the pH to 6.9-7.1, and fix the volume to 20L.

8. 用 Millipak 40 或类似过滤器除菌过滤到使用容器中。

   Use a Millipak 40 or similar filter to remove bacteria and filter into a container for use.

【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 S4 CHO 中。

Most cell lines can be directly domesticated to S4 CHO.

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 %（S4 CHO：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% (S4 CHO: 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%（S4 CHO：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% (S4 CHO: 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% S4 CHO 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% S4 CHO 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% S4 CHO 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% S4 CHO, 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度很低，可考虑从 10：90（S4 CHO：原培养基）体积比配制的混合培养基起，缓慢增加 S4 CHO 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, you can consider slowly increasing the ratio of S4 CHO to 25: 75, 50: 50, 75: 25, 100: 0 from the mixed medium prepared with a volume ratio of 10: 90 (S4 CHO: 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% S4 CHO + 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% S4 CHO +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 1.0~1.5×10^7cells/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 S4 CHO；

Prepare 15 ml sterile centrifuge tube and add 2~5mL S4 CHO；

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 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 S4 CHO 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 S4 CHO, 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 S4 CHO 重新悬浮，转移至 125 ml 摇瓶中；

Slowly pour out the supernatant, resuspend with 20~30 ml preheated S4 CHO, and transfer to a 125 ml shake flask；

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

Place it in a shaking incubator with 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】

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、有效期或复验期 Storage condition, validity period or retest date】

上海生产基地，S4 CHO 干粉培养基 A 组分、S4 CHO 干粉培养基 B 组分、S4 CHO 干粉培养基 C：2~8℃、避光储存，有效期为 12 个月。

Shanghai production base, S4 CHO dry powder medium - component A, S4 CHO dry powder medium - component B, and S4 CHO dry powder medium - component C: 2℃ to 8℃, protect from light; validity period: 12 months.

无锡生产基地，S4 CHO 干粉培养基 A 组分、S4 CHO 干粉培养基 B 组分：2~8℃、避光储存，复验期为 12 个月。S4 CHO 干粉培养基 C：2~8℃、避光储存，有效期为 12 个月。

Wuxi production base, S4 CHO dry powder medium - component A, and S4 CHO dry powder medium - component B: 2℃ to 8℃, protect from light; retest date: 12 months. S4 CHO dry powder medium - component C: 2℃ to 8℃, protect from light; validity period: 12 months.

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