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

V146-01

【产品名称 Product name】**S4 CHO 粉末培养基 S4 CHO powder medium**

【主货号 Main Art. No.】MP016 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 basal medium with no animal-derived ingredients, no serum ingredients and no protein ingredients, which is suitable for batch culture, fed-batch culture and perfusion culture during the development and production of therapeutic protein products using Chinese Hamster Ovary (CHO) cells. S4 CHO Medium is free of hypoxanthine, thymine, and L-glutamine, and contains a small amount of hydrolysate of yeast and soybean origin. 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 specifically designed to enhance the growth and productive properties of CHO cells.

- 本产品需要和基础培养基 B 粉末混合使用；

  This product needs to be used with Basal Medium B powder;
- 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 adding insulin, the recommended addition concentration is 0.5-10mg/L.

【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)

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1. 准备配液体积 90%左右的超纯水（20~30℃）；

   Prepare ultrapure water (20~30℃) with about 90% of the volume of the prepared liquid;

2. 加入 S4 CHO 培养基粉末 26.77g，搅拌 20 min，溶解完全。

   Add S4 CHO medium powder 26.77g, stir for 20 min, dissolve completely.

3. 另外准备 25mL 0.4M NaOH 溶液，加入基础培养基 B 粉末 1.48g，搅拌 10min，溶解完全，记为 B 溶液；

   Prepare 25mL of 0.4M NaOH solution, add 1.48 g of basal medium B powder, stir for 10 min, dissolve completely, and record it as B solution;

4. 依次加入 B 溶液、4mg 酚红钠、1.6g 碳酸氢钠，继续搅拌 20 min；

   Add B solution, 4mg of sodium phenol red, 1.6g of sodium bicarbonate in turn, and continue stirring for 20 min;

5. 采用 6M HCl 调节 pH 至 7.00~7.40；

   Adjust the pH to 7.00~7.40 with 6M HCl;

6. 定容，搅拌 5~10 min；

   Constant volume, stirring for 5~10 min;

7. 用 0.22μm 过滤器除菌过滤。

   Sterilize and filter with a 0.22μm filter.

【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 do not require any domestication when using this product, they can be inoculated directly into this medium and passaged for more than three times. For some cell lines, the use of this series of medium may require domestication, 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 培养基。

Most cell lines can be domesticated directly into S4 CHO 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。

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.

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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°C和 6% CO_2 培养。细胞培养 3~4 天达到 1×10^6 cells/mL 以上，传代；

When the cell line was cultured in the original medium until the middle of the exponential growth period and the cell viability was >90%, the cell line was inoculated into the mixed medium prepared with 50%:50% (S4 CHO: original medium) volume ratio, the inoculation density was 3~5×10^5 cells/mL, and the cells were cultured at 36.5°C and 6% CO_2. Cells were cultured for 3~4 days to reach more than 1×10^6 cells/mL and passaged;

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 mixed medium prepared at 75%:25% (S4 CHO: original medium) volume ratio at an inoculum density of 3~5 × 10^5 cells/mL and cultured at 36.5°C and 6% CO_2. The cells were cultured for 3~4 days to reach more than 1×10^6 cells/mL and passaged;

将该细胞接种到 100% S4 CHO 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；

Inoculated the cells into 100% S4 CHO medium at a density of 3~5 × 10^5 cells/mL and cultured at 36.5°C and 6% CO_2. The cells were cultured for 3~4 days to reach more than 1×10^6 cells/mL and passaged;

在 100% S4 CHO 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% S4 CHO medium, passaging for at least 2~3 generations with normal and stable doubling time and cell viability >90% indicated that the cell line domestication was completed;

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, we 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 培养基+10% 二甲基亚砜（DMSO）混合液，2~8℃预冷（DMSO 稀释时会释放热量）；

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Prepare freezing solution on the ultra-clean bench: 90% S4 CHO medium + 10% Dimethyl Sulfoxide (DMSO) mixture, pre-cooled at 2~8℃ (DMSO will release heat when 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: 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；

Centrifuge the cell culture fluid 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, 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°C 温水，用于解冻细胞；

Prepare 36.5°C warm water to thaw cells;

②准备 15 ml 无菌离心管，加入 2~5mL 的 S4 CHO 培养基；

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

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③从液氮罐中取出冻存管，迅速在 36.5°C 温水中解冻细胞；

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

Slowly pour out the supernatant, re-suspend it using 20~30 mL of pre-warmed S4 CHO medium and transfer it 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 in a shaker at 36.5℃, 8% CO_2, 80% humidity, 110~130rpm;

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 passaged.

【细胞传代 Cell passaging】

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。

Cells were subcultured according to the density of 5E5~6E5, counted every 2-3 days, and subcultured. After the first three passages, the volume remained unchanged to restore the cell viability. After the cell viability returns to normal, reaching more than 90%, it is amplified at the density of 5E5 ~6E5 until the required seed volume is reached. The standard of normal seed state is that the viability is more than 95%, the cell morphology is regular and round, and the growth doubling time is 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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