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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 Medium for Animal-Cells

V179-00

【产品名称 Product name】Maxgrow 201

【货号 Art. No.】MP030-100g-02、MP030-1kg-02、MP030-5kg-02、MP030-10kg-02、MP030-25kg-02

粉末包装 Powder packaging

【产品说明 Product description】

Maxgrow 201 是一款专门用于中国仓鼠卵巢（CHO）细胞生长和在 CHO 细胞中生产重组蛋白的化学成分限定粉末培养基。该培养基不含 L-谷氨酰胺，可用于谷氨酰胺合成酶，或 GS 系统™的细胞培养。应用于其他细胞类型，在使用前可添加低水平的 L-谷氨酰胺或 GlutaMAX™。通常情况下，在 Maxgrow 201 培养基中推荐添加 8g/L 葡萄糖，不会造成潜在的乳酸积累问题。

Maxgrow 201 is a chemically-defined powder medium designed especially for the growth of Chinese Hamster Ovary (CHO) cells and production of recombinant protein in CHO cells. The medium is supplied without L-glutamine for use in Glutamine Synthetase, or GS System™. For other applications, add low levels of L-glutamine or GlutaMAX™ prior to use. The recommended glucose concentration to add into Maxgrow 201 Medium is 8g/L under typical culture conditions and won't cause potential lactic acid accumulation problem.

【使用指南 User guide】

本产品仅供科研或生产制造；不能直接用于人体或治疗用途。

This product is for research or for further manufacturing use. This product is not intended for human or therapeutic use.

【配制指南 Preparation guide】

适用于 Maxgrow 201 粉末包装（以 1L 为例）

Suitable for Maxgrow 201 powder packaging (take 1L as an example)

1.准备配液体积 80%的超纯水（常温 20~30℃）；

1. Prepare ultrapure water with 80% of the volume of the solution (room temperature 20~30℃);

2.打开搅拌器，加入 1.0 g/L P188；

2. Turn on the mixer and add 1.0 g/L P188;

3.加入 8.0 g/L D-葡萄糖；

3. Add 8.0 g/L D-glucose;

4.加入 9.4 g/L Maxgrow 201 干粉；

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4. Add 9.4 g/L Maxgrow 201 powder;

5.充分搅拌 20 分钟至全部溶解;

5. Stir for 20 minutes until completely dissolved;

6.加入 1.83 g/L 碳酸氢钠;

6. Add 1.83 g/L sodium bicarbonate;

7.加入 2.3 g/L 氯化钠;

7. Add 2.3 g/L sodium chloride;

8.充分搅拌 20 分钟至全部溶解;

8. Stir for 20 minutes until completely dissolved;

9.将 pH 调整到 6.9-7.1;

9. Adjust pH to 6.9-7.1;

10.加入 15 ml/L 1M HEPES;

10. Add 15 ml/L 1M HEPES;

11.定容, 测定渗透压, 无菌过滤, 2-8℃ 保存。

11. Constant volume, measure osmotic pressure, sterile filter, and store at 2-8℃.

【细胞培养 Cell culture】

①建议细胞传代密度: $0.3\sim0.5\times10^6$ cells/mL, 细胞密度至 $1.5\sim3.5\times10^6$ cells/mL 时进行传代操作。

Recommended cell passage density: $0.3\sim0.5\times10^6$ cells/mL, when the cell density reaches $1.5\sim3.5\times10^6$ cells/mL, the passage operation is carried out.

②温度 Temperature: 37℃。

③CO₂: 5-8%。

【细胞驯化 Cell domestication】

通常情况下, CHO-K1, CHO-K1 SV, CHO-S 等 CHO 细胞可以直接从无血清培养基或化学限定培养基切换到 Maxgrow 201 中进行培养。

In many cases, CHO cells such as CHO-K1, CHO-K1 SV, CHO-S may be subcultured from a serum-free medium or chemically defined medium directly into Maxgrow 201.

1.将 Maxgrow 201 培养基装到合适的培养容器中并平衡至 37℃ 和 5% CO₂。

1. Dispense Maxgrow 201 medium into an appropriate culture vessel and equilibrate to 37° C and 5% CO₂.

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2.将 CHO 细胞以 $3-5 \times 10^5$ cells/mL, 从无血清培养基或其他化学限定培养基接种到 Maxgrow 201 中。细胞应处于对数生长阶段, 不少于 90% 的活率。

2. Passage CHO cells from serum-free culture or other chemically defined media into Maxgrow 201 at $3 - 5 \times 10^5$ viable cells/mL. It is important that cells should be in the logarithmic phase of growth with at least 90% viability before passaging.

3.在 37°C 和 5% CO₂ 下培养细胞, 直到活细胞密度大于 1×10^6 cells/mL, 最好是 $1.5 - 3.5 \times 10^6$ cells/mL。

3. Incubate cultures at 37° C and 5% CO₂ until the viable cell density greater than 1×10^6 cells/mL, preferably $1.5 - 3.5 \times 10^6$ cells/mL.

4.以 $3 - 5 \times 10^5$ cells/mL 接种密度, 传代培养到 Maxgrow 201 培养基中。

4. Subculture into Maxgrow 201 medium at $3 - 5 \times 10^5$ cell/mL seeding density.

5. 使用 Maxgrow 201 传代细胞至少 3 次, 以实现完全适应并确保最佳性能。

5. Maintain cells in Maxgrow 201 for at least three passages to allow complete adaptation and assure optimum performance.

6.培养过程需补充 L-谷氨酰胺, 推荐 L-谷氨酰胺添加浓度从 1mM 到 4mM, 以获得最佳效果。

6. For the culture required L-Glutamine supplementation, titrate L-Glutamine concentration from 1mM to 4mM for the best results.

【细胞冻存 Cell cryopreservation】

冻存液: 45% Maxgrow 201 + 45% 条件培养基 + 10% DMSO。不需要蛋白质或其他添加物。

Freezing solution: 45% Maxgrow 201 + 45% condition medium + 10% DMSO. No proteins or other additions are necessary.

收集细胞于离心管中, 800-1000rpm 离心 5 分钟后倒掉上清;

Collect the cells in a centrifuge tube, centrifuge at 800-1000rpm for 5 minutes and discard the supernatant;

冻存液重悬细胞, 每支冻存管储存 $1.0 \times 10^7 - 1.5 \times 10^7$ 的细胞量, 放入冻存盒中, -80°C 过夜后转入液氮罐中永久保存。

Resuspend the cells in the freezing solution, store $1.0 \times 10^7 - 1.5 \times 10^7$ cells in each cryovial, put them in a freezing box, and transfer them to a liquid nitrogen tank for permanent storage after overnight at -80 °C.

【细胞复苏 Cell resuscitation】

①准备 37 °C 温水, 液氮中拿取细胞立即放入温水中, 快速融化细胞;

① Prepare 37 °C warm water, take the cells from liquid nitrogen and put them in warm water immediately to quickly thaw the cells;

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② 直接将 1 支冻存管的细胞重悬在预热的 Maxgrow 201 中，细胞密度控制在 3E5-5E5 之间，转移至 125 ml 摇瓶中培养；

② Directly resuspend the cells in one cryopreservation tube in pre-warmed Maxgrow 201, control the cell density between 3E5-5E5, and transfer to a 125 ml shake flask for culture;

③ 放置于 37°C，5% CO₂ 摇床中培养；

③ Culture in a shaker at 37°C with 5% CO₂;

④ 培养 2-3 天后，对细胞进行计数传代。

④ After 2-3 days of culture, count and passage the cells.

【细胞传代 Cell passage】

按照 3E5 ~5E5 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。种子状态正常的标准：活力大于 90%，细胞形态规则圆整，生长倍增时间正常。

Cells were passaged at the density of 3E5-5E5, counted every 2-3 days, and passaged. For the first 3 passages, the volume was unchanged to restore cell viability. The standard of normal seed status: the vigor is more than 90%, the cell shape is regular and round, and the growth doubling time is normal.

【储存和复验期 Storage and retest period】

Maxgrow 201 粉末包装：2~8°C 避光储存，复验期为 24 个月。

Maxgrow 201 powder packaging: store in dark at 2~8°C, and the retest period is 24 months.

【生产企业信息 Manufacturer information】

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