# Media C-01

## High-Performance Culture Medium for Animal-Cells

## Product Instruction Manual

**DUONING**

**High-Performance Culture Medium for Animal Cells**

## Product Name: Media C-01
## Main Product No.: LB003; Liquid packaging

### Product Description

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

### Cell Culture

1) Suggested cell inoculation density: 0.3-1.0 x $10^6$ cells/mL.
2) Temperature: 36.5℃
3) $CO_2$: 6-8%

### Cell Adaption

Most cell lines use this product without any adaption and can be directly inoculated into this medium and passaged for more than three times. For some cell lines, adaption may be used when using this series of medium, and the specific steps are as follows:

**1) Direct adaption**

Most cell lines can be directly adapted to Media C-01.
Cell inoculation density: 3.0~8.0×$10^5$ cells/mL
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 adapted.

**2) Continuous adaption**

- 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-01: original medium), and the inoculation density was 3~5×$10^5$ cells/mL, and it was cultured at 37°C and 6% $CO_2$. The cells were cultured for 3~4 days to reach more than 1×$10^6$ cells/mL, and then subcultured.
- Cells were inoculated into a 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 37°C and 6% $CO_2$. The cells were cultured for 3-4 days to reach more than 1×$10^6$ cells/mL, and then subcultured.
- The cells were inoculated into 100% Media C-01 with the inoculation density of 3-5× $10^5$ cells/mL, and cultured at 37℃ and 6% $CO_2$. The cells were cultured for 3-4 days to reach more than 1× $10^6$ cells/mL, and then subcultured.
- In 100% Media C-01, 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 adapted.
- When adopting this adaption procedure, if the cells still grow slowly or have low activity, we can consider slowly increasing the ratio of Media C-01 to 25: 75, 50: 50, 75: 25, 100: 0 from the mixed medium prepared

with a volume ratio of 10: 90 (Media C-01: original medium). Or the cells are collected by centrifugation during the process and subcultured again.

## Cell Cryopreservation

1) Prepare frozen solution on the super clean workbench : 90% Media C-01 +10% dimethyl sulfoxide (DMSO) mixed solution, precooling at 2-8°C (heat will be released when DMSO is diluted);
2) Frozen cell suspension: in the exponential growth period, the density is greater than $1.5\times10^6$ cells/mL, and the viability is greater than 95%. Generally, it is recommended that the frozen storage density is 1.0~ 1.5x $10^7$ cells/ml;
3) Cell suspension was centrifuged at 800 rpm for 5 min;
4) Slowly pour out the supernatant, resuspend the cells with cryopreservation solution, the cryopreservation density is $1.0\sim1.5\times10^7$ cells/mL, and transfer the cells to a sterile cryopreservation tube;
5) 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:
    - Freeze at 4 ℃ for 30 min;
    - Freeze at -20 ℃ for 2-4 h;
    - Freeze at – 80 ℃ overnight;
    - Transfer frozen cells to liquid nitrogen tank for long-term storage

## Cell Recovery

1) Prepare a 36.5 °C warm water to thaw frozen cells;
2) Prepare 15 ml sterile centrifuge tube and add 2~5mL Media C-01;
3) Take out the frozen tube from the liquid nitrogen tank and quickly thaw frozen cells in 36.5°C warm water;
4) After wiping the cryopreservation tube with 75% ethanol, open the cryopreservation tube in the aseptic ultraclean workbench, transfer the cell suspension to a 15 ml centrifuge tube containing 2-5 mL of Media C-01, mix well, centrifuge at 800 rpm for 5 minutes;
5) Slowly pour out the supernatant, resuspend with 20-30 ml preheated Media C-01, and transfer to a 125 ml shake flask;
6) Place it in a shaking incubator with 6-8% $CO_2$, 110~130 rpm, at 36.5 °C for culture;
7) After 2-3 days of culture, the cells were counted and subcultured.

## Cell Passage

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.

## Storage and Validity Period

Media C-01 liquid packaging: 2 °C to 8 °C, protect from light; Shelf life: 12 months.

## Manufacturer Information

Company Name: Wuxi Duoning Biotechnology co.,Ltd

Address: No.2-1, No.2-2, Xinji Road, Singapore Industrial Park, Wuxi
Tel: 0510-85956600
Website: www.duoningbio.com/en

## Duoning Biotechnology Group

marketing@duoningbio.com

www.duoningbio.com/en

6/F manulife place 348kwun tong road kl, HongKong, PRC.