Cryopreservation of CHO Cells Using Serum-Free Media

Yoo Kang Kim[1], Hong Woo Park[2] and Tae Boo Choe[1]
[1]Dept. of Microbial Engineering, Konkuk University
TEL: +82-2-450-3523, FAX: +82-2-3436-5594
[2]Dept. of Chemical Engineering, Hanyang University

Abstract

During routine maintenance, animal cell lines are commonly cryopreserved in growth medium containing serum with 10% DMSO. But, in case of bioprocess under the serum-free condition, including cultivation of cell lines and producing of pharmaceuticals, the cryopreservation should be executed without serum to prevent a cross-contamination. This experiments were performed to investigate the effects of the serum-free cryopreservation on the CHO cells. To improve the survival rates of the cryopreserved CHO cells in serum-free condition, first, the effects of permeable and non-permeable additives for substitute serum on cell viability were investigated.[1)] The combination of 10% DMSO and 0.3M raffinose in MEM-α without serum indicated 76% of cell viability. However, it did not reach the survival rates (more than 95%) of the conventional cryopreservation. In the second, to evaluate the cryopreservative ability of the serum-free medium (SFM) we compared viability of the CHO cells cryopreserved in the SFMs (Sigma C5467, C4726, and C1707, JBI SF486 and PF486), the cryoprotectant (Genenmed CAN-1000) and the MEM-α with serum. All solution contained 10% DMSO. As a result of the comparison, cryopreserved cells in the SFMs showed over 95% of viability and appeared predominant viability better than cryoprotectant CAN-1000. Finally, we assessed the stability of the CHO cells in the long-term cryopreservation (LTC) using SFM. The cryopreserved CHO cells were thawed every three months to estimate the cell viability and the recovery rates. Then, real-time RT-PCR analyzed the inserted CHO DHFR gene. All results for the LTC appeared the same stability as the serum containing cryopreservation. In the conclusion, it could be seen that the LTC in the SFM can substitute for serum using methods in the bioprocess proceeded by CHO cells for more than two years.

Reference

1. Kelbe, RJ. & Mancuso, MG., Identification of new cryoprotective agents for cultured mammalian cells (1983), In Vitro, Vol (19), 167-170.