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MODIFIED METHOD OF FREEZING SUBCULTURED CELL LINES

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Long-term storage of cell lines is a crucial component of modern cell biology, oncology, and biomedical research. Continuous passaging of cells increases the risk of genetic drift, phenotypic alterations, and contamination. Cryopreservation reduces these risks by maintaining cells in a metabolically inactive state. In addition, cryopreservation ensures genetic stability, experimental reproducibility, and continuity of cell-based studies.

During the freezing process, cells are primarily damaged by intracellular ice crystal formation, osmotic stress during cooling and thawing, destabilization of cell membranes, and protein denaturation. Classical cryopreservation methods are based on controlled-rate freezing and the use of cryoprotective agents, predominantly dimethyl sulfoxide (DMSO). However, depending on the cell type, growth conditions, and laboratory capabilities, standard protocols often require modification. The application of isopropanol-based passive cooling systems enables controlled cooling rates without the need for complex equipment while preserving cell viability.

In the present study, a modified method for freezing subcultured cell lines is described, involving stepwise temperature reduction using isopropanol and an optimized concentration of DMSO. The proposed approach represents an efficient, reproducible, and technically accessible method for long-term cell preservation prior to storage in liquid nitrogen.

Subcultured cells were harvested during the logarithmic growth phase to ensure maximal viability. Adherent cells were detached using “Versene” solution, while suspension cells were collected by centrifugation. A complete culture medium containing a final concentration of 5% (v/v) DMSO was prepared. This concentration reduces cytotoxic effects compared to the conventional 10% DMSO while providing sufficient cryoprotective efficacy.

The cell suspension was aliquoted into sterile cryovials, which were placed into an isopropanol-based freezing container, allowing a gradual cooling rate of approximately −1°C per minute. The samples were maintained at −80°C for 24 hours, after which the cryovials were transferred to liquid nitrogen (−196°C) for long-term storage.

In conclusion, this modified cryopreservation method offers several advantages, including lowering the freezing point of intracellular water, limiting ice crystal formation, and stabilizing cell membranes. Overall, the described protocol provides an effective and reliable approach for the cryopreservation of subcultured cell lines.