Cryopreservation and Storage of Cell Lines

Elizabeth Fashola-Stone, Marc Jones and Bryan Bolton *European Collection of Cell Cultures, Health Protection Agency, Porton Down, Wiltshire, UK*

Benefits that Cryopreservation Offers

Cryopreservation is invaluable when dealing with cells of limited life span. It allows cells to be stored at ultra-low temperature for future use without having to resort to the continuous cultivation of cell lines. Other main advantages include:

- Reduced risk of microbial contamination
- Reduced risk of cross contamination with other cell lines
- Reduced risk of genetic drift and morphological changes
- Work conducted using cells at a consistent passage number
- Reduced costs

The Requirements for Successful Cryopreservation

A large amount of development work has been undertaken to ensure successful cryopreservation and resuscitation of a wide variety of cell lines of different cell types. The basic principal of cryopreservation is to slow freeze and quick thaw. The most reliable and reproducible way to achieve a slow freeze at a rate of -1°C to -3°C per minute is with the use of a programmable rate controlled freezer. The cost in acquiring such equipment is often beyond the budget for the majority of research laboratories. An alternative approach is to freeze passively by keeping the ampoules for 24 hours in a Nalgene Mr Frosty (Sigma Product No. C1562) filled with isopropyl alcohol at –80°C.

Cryropreservation also depends upon the use of a high concentration of serum/protein (>20% should be used and in many cases serum is used at 90%) and cryoprotectants such as dimethyl sulphoxide (DSMO, Sigma Product No. D2650) or glycerol (Sigma Product No. G2025). Both cryoprotectants help to prevent the cells from rupturing due to the formation of ice crystals. DMSO is the most common cryoprotectant used at a final concentration of 10%, however, this is not always appropriate because DMSO induces differentiation in some cell lines (e.g. HL60, ECACC Product No. 98070106-1v1). In such cases, glycerol is often used as the alternative (refer to ECACC data sheet for details of the correct cryoprotectant for a particular cell line). It is essential that immediately prior to cryopreservation cultures should be healthy with a viability of >90% and in the log phase of growth. The latter parameter can be achieved by using pre-confluent cultures (i.e. cultures that are below their maximum cell density).

Ultra-Low Temperature Storage of Cell Lines

Following controlled rate freezing, cells can be cryopreserved in a suspended state for an indefinite period provided a temperature of less than –135°C is maintained. ECACC strongly discourages the idea of long term storage at -80°C. Such ultra-low temperatures can only be attained by specialised electric freezers or more usually by immersion in liquid or vapour phase nitrogen. The advantages and disadvantages of each are summarised below:

Table 1. Comparison of ultra-low temperature storage methods for cell lines.

Methods	Advantages	Disadvantages
Electric (-135°C) Freezer	• Ease of maintenance • Steady temperature • Low running costs	• Requires liquid nitrogen back-up • Mechanically complex • Storage temperatures high relative to liquid nitrogen
Liquid Phase Nitrogen	• Steady ultra-low (-196°C) temperature • Simplicity and mechanical reliability	• Requires regular supply of liquid nitrogen • High running costs • Risk of cross-contamination via the liquid nitrogen
Vapour Phase Nitrogen	• No risk of cross-contamination from liquid nitrogen • Low temperatures achieved • Simplicity and reliability	• Requires regular supply of liquid nitrogen • High running costs • Temperature fluctuations between - 135°C and - 190°C