# Efficient cryopreservation of human ES and iPS cells in a chemically defined, cGMP produced, serum-, xeno-, and DMSO-free freezing medium

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Human pluripotent stem cells (hPSCs) show great promise in regenerative medicine, drug discovery, as well as importance for basic research. It is therefore a priority to establish efficient, user-friendly, and robust methods for bulk hPSCS cryopreservation. Thus far, obtaining good survival after thawing is problematic and, furthermore, current slow-freezing protocols result in hPSCs with a tendency to spontaneously differentiate.

We have developed a novel, current good manufacturing practice (cGMP) produced, chemically defined, xeno- and dimethyl sulfoxide (DMSO)-free cryopreservation medium denoted FREEZEstem™ for cryostorage and banking of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). The hPSCs were frozen in FREEZEstem™ and compared to a commercial cryomedium containing 10% DMSO and a standard cryomedium with 10% fetal calf serum and 10% DMSO. Viability after thawing, toxicity of the freezing media as well as the impact of different thawing solutions was assessed. The cellular phenotype was evaluated using immunocytochemistry or flow cytometry analysis and the proliferation and differentiation potential were investigated.

We found that directly after thawing, the same or a higher number of hESC and iPSC colonies were detected for cells frozen in FREEZEstem™ and these cells were less sensitive to the thawing solution. Abundant expression of stem cell markers, high proliferation rate and extensive differentiation potential were detected for hPSCs frozen in all three cryomedia. Importantly, we have optimized the protocol for single-cell freezing and, thus, is ideal for use in a hPSC culture system which supports single-cell passaging such as the LN-521™ matrix.

FREEZEstem™ not only has the advantage of being cGMP manufactured and DMSO-free, it is also a user-friendly, robust freezing medium with very low toxicity enabling total control over hPSC cultures, thus offering an excellent, simple option for banking human ES and iPS cells.