



In order to evaluate the integrity of the cell membrane of HL-60 cells during heating a trypan blue dye exclusion experiment was performed. Aliquots of approximately 3.0×10^4 HL-60 cells in culture medium were heated to 37, 50, 60, 65 and 70°C respectively for 3 min in the presence of 0.2% (w/v) trypan blue dye and allowed to cool to room temperature for 3 minutes. A 10 μ l aliquot of the heated cell suspension was subsequently analyzed using a TC20™ automated cell counter. Cells with the ability to exclude trypan blue were considered as retaining their cell membrane integrity whereas dye stained cells were identified as cells with disrupted cell membranes. The data demonstrated that the cell membrane of the HL-60 cells remain intact during heating at 50°C (the temperature at which the ITDRF_{CETSA} experiments were conducted) since no statistically significant decrease in dye exclusion was observed compared to the initial dye exclusion capability (control) of the HL-60 cells. In addition, the data demonstrated that the HL-60 cell membranes are not disrupted unless temperatures above 60°C are reached. This is in agreement with our previous study where several other cell lines demonstrated loss of cell membrane integrity at temperatures above 60°C¹. Data are given as the average \pm standard deviation ($n \geq 4$).