cells using twofold, i.e. 1:2, 1:4 and 1:8, media dilutions instead of the "usual" 1:5 and 1:10 splitting routines results in a workflow that is in synchrony with the doubling interval[6] of the individual cell line used and may contribute to improved assay results. Although this still doesn't prevent the experimenter from sometimes ending up at different cell densities at harvest, it may improve the chances of harvesting (enough) cells in their exponential growth phase. When for example harvesting 7,000,000 exponentially growing cells in sub-confluent state from a 162 cm^2 flask chances are high to also assume that every cell count > 2,500,000 cells harvested from less confluent cultures should primarily consist of exponentially growing cells and be (working) fine. Any cell count below and above these arbitrarily chosen lower and upper margins should be considered inappropriate for further processing and experimentation. In addition, to ensure proper and reproducible functional assay responses of a cellular system, it is very important – and very easily done – to always record and document the cell numbers obtained when subculturing cells on a regular basis. If undetected, any deviation can seriously affect experimental results. Furthermore, this simple procedure also detects any drift in cell line activity due to ageing processes and/or contamination as added value.

5 Cells as Reagents (Frozen Cells)

The use of frozen cells[7] can be very helpful to free the lab from the uncertainties of continuously ongoing routine cell culture. Although this has become a mainstay in the screening campaigns of many pharma companies (Cawkill and Eaglestone 2007; Zaman et al. 2007), surprisingly little detailed information regarding the actual preparation and resuscitation of cells to be used as reagents has been published. Today, the vast majority of the cell-based assays developed in our department are making use of this straightforward, highly robust and reproducible approach providing the experimenter with almost the same experimental freedom that a biochemical test system brings about in addition to delivering high-quality, functional assay data. In fact, what started years ago as a measure to primarily free the experimenter from routine cell culture efforts and assay cell provision (Bergsdorf et al. 2008) and with rather simple screening assay designs has developed into a veritable success story today involving bulk frozen cell provision by CROs and an almost 100% routine use of frozen cells in complex functional

[6] See Freshney (2000b).

[7] The term frozen cell describes the use of freshly resuscitated cells shortly after their recovery from liquid nitrogen storage and should not to be mistaken for the use of division-arrested cells, as exemplified/described by Digan et al. (2005).