Stability of resazurin in buffers and mammalian cell culture media

The utility of a ferricyanide/ferrocyanide system used in the AlamarBlue(TM) (Serotec, Oxford, UK) vital dye to inhibit the reduction of resazurin by mammalian cell culture media is questioned. Resazurin was found to be relatively stable when dissolved in phosphate-buffered saline (PBS). The use of HEPES resulted in a huge immediate dye reduction, which was significantly enhanced by exposure to diffuse light from fluorescent tubes in the laboratory 8 h per day. The reduction of resazurin by various cell culture media was time and temperature dependent, and it was significantly enhanced by prolonged exposure to the Light in the laboratory. A pronounced reduction of resazurin was observed in the RPMI 1640 medium, which contains 1 mg/L of reduced glutathione. No significant differences in resazurin reduction were observed between McCoy's 5A medium, Dulbecco's Modified Essential medium, and Ham's nutrient mixture F-10 and F-12. Fetal calf serum (5-20%) slightly decreased resazurin reduction during the first 2 days of incubation. The reduction of resazurin by mammalian cell culture media do not appear to be problematic under normal culture conditions, and it is primarily dependent upon the presence of photocatalytic or reducing agents.