Contribuições / Contribuciones / Contributions

Por um Desenvolvimento Ambiental Sustentado no Próximo Milênio
Por un Desarrollo Ambiental Sustenible en el Próximo Miléncio
For a Sustainable Environmental Development in the Next Millenium

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IMPLEMENTATION OF A VIABILITY/CITOTOXICITY ASSAY IN
Tetrahymena pyriformis USING FLUORESCENT PROBES

Nicolina Dias and Nelson Lima
Centro de Engenharia Biológica-IBQF, Universidade do Minho,
Campus de Gualtar, 4710-057 Braga, Portugal

In vitro cytotoxicity assays as been widely used for medical purposes, particularly in human
tumour cell lines studies, in characterizing the function of T lymphocytes, being an essential
tool to monitorize immunocompetence of cancer and infectious diseases. A broad variety of
techniques have been developed to evaluate cell death, using markers, either retained by
living cells or released from lysed ones. Chromium (Cr)-release assay was the standard
method for cytotoxicity evaluation, however it requires the handling of radioactive
compounds that subjects laboratory staff to various hazards. As an alternative, the use of
fluorogenic esterase derivatives substrates to stain live cells has been widely used as
cytoplasmic markers and viability probes.
4'5'-Bis(N'N'-bis (carboxymethyl) aminomethyl fluorescein acetoxymethyl ester
(AM) is a non-fluorescent, lipophilic substrate that diffuses passively into cells and is
converted by intracellular esterase activity to calcein, a fluorescent molecule that produces an
intense green at 530 nm. Once hydrolysed highly negative charged free calcein is lipid-
insoluble, being effectively trapped inside cells that maintain their membrane integrity. As
the probe is rapidly released from membrane-damaged cells, only intact cell stain green.
Dead cells stain red by the addition of a second dye Ethidium homodimer-1 is a cell
impermeable fluorochrome that passes through compromised membranes, strongly binds to
nucleic acids and emits red fluorescence at 617 nm.
Ciliated protozoa play an essential role in the purification process of aerobic and anaerobic
biological treatment systems, by removing dispersed bacteria which are responsible for high
turbidity in the final effluent. In the other hand protozoa are very sensitive to environmental
changes that makes them suitable to studies as biological indicators of pollution. The ciliated
Tetrahymena pyriformis was used in this work as a model organism for the assessment of
protozoa viability exposed to toxicants. Two distinct populations - green live cells and red
dead cells - enable the user to quickly quantify viability vs. cell death. Data analysis is then
possible to performed by advanced imaging techniques, such as flow cytometry or image
analysis. It is hoped that this new method will be useful as a fast, non-toxic to cells, easy and
low-cost assay to complement other in vitro assays.