DISCOVERY SERVICES

DRUG DISCOVERY

Cytotoxicity

Background

The predictive value of *in vitro* cytotoxicity tests is based on the idea of 'basal' cytotoxicity – that toxic chemicals affect basic functions of cells which are common to all cells, and that the toxicity can be measured by assessing cellular damage. The development of *in vitro* cytotoxicity assays has been driven by the need to rapidly evaluate the potential toxicity of large numbers of compounds, to limit animal experimentation whenever possible, and to carry out tests with small quantities of compound. Evidence for the utility of *in vitro* cytotoxicity tests[1,2,3] has led many pharmaceutical companies to screen compound libraries to remove potentially toxic compounds early in the drug discovery process. Early identification of toxic effects can help project teams prioritize between chemical series and identify Structure-Toxicity Relationships[3] to reduce cost downstream.

Assay Principle

There are 3 basic parameters upon which these measurements are based. The first assay type is the measurement of cellular metabolic activity. An early indication of cellular damage is a reduction in metabolic activity. Tests which can measure metabolic function measure cellular ATP levels or mitochondrial activity (via MTS metabolism). Another parameter often tested is the measurement of membrane integrity. The cell membrane forms a functional barrier around the cell, and traffic into and out of the cell is highly regulated by transporters, receptors and secretion pathways. When cells are damaged, they become 'leaky' and this forms the basis for the second type of assay. Membrane integrity is determined by measuring lactate dehyrogenase (LDH) in the extracellular medium. This enzyme is normally present in the cytosol, and cannot be measured extracellularly unless cell damage has occurred. Other assays measure the uptake of fluorescent dye (ethidium DI) normally excluded from intact cells. It has been shown that changes in metabolic activity are better indicators of early cell injury, and that effects on membrane integrity are indicative of more serious injury, leading to cell death[2]. The third type of assay is the direct measure of cell number, since dead cells normally detach from a culture plate, and are washed away in the medium. Cell number can be measured by direct cell counting, or by the measurement of total cell protein or DNA, which are proportional to the number of cells.

Key Features of the Assay

- low compound consumption
- multiple assays: DNA, ATP, MTS, membrane integrity
- six cell lines from different tissues available: liver, kidney, lung, brain, testis, ovary

Assay Applications

- prioritization of hits based on cytotoxicity
- assessment of compound library for cytotoxicity

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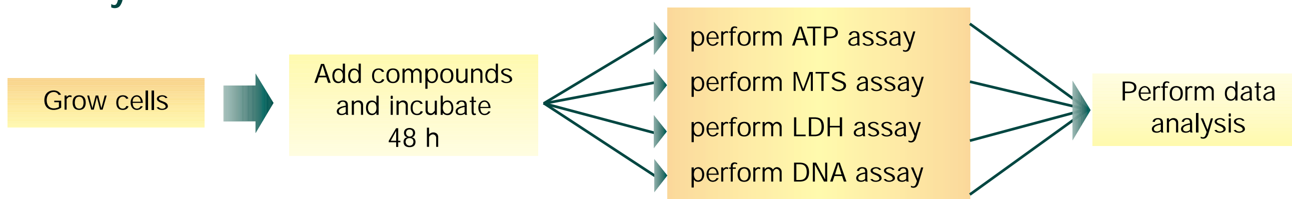
Assay Protocol



ATP Assay

- add dye
- mix and incubate 10 min
- measure luminescence intensity

MTS Assay

- add dye
- mix and incubate 2 h
- measure absorbance intensity

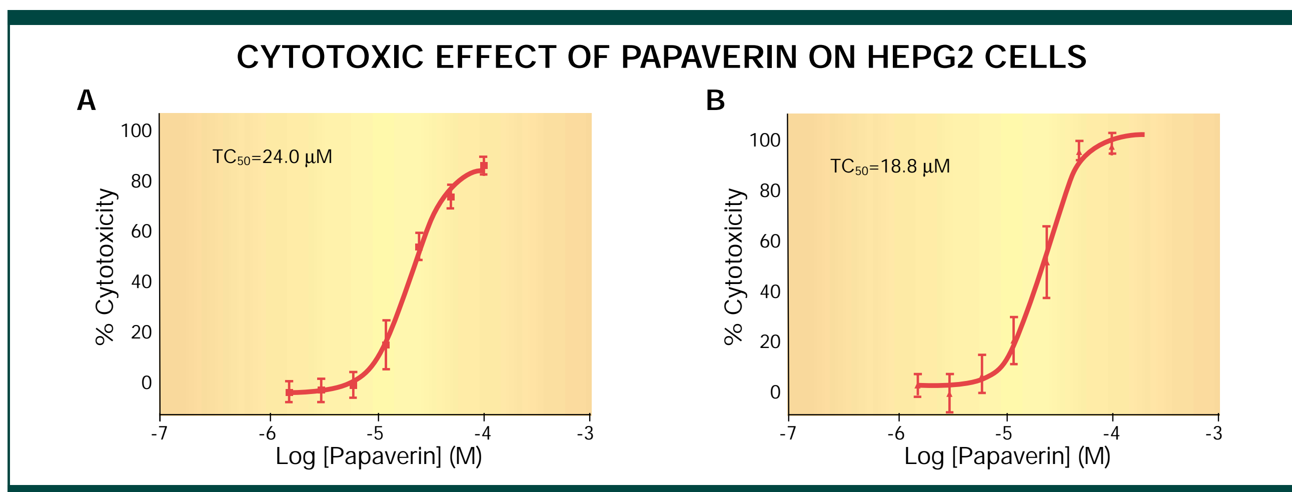
DNA Assay

- freeze plate for 24 h
- thaw then add dye
- incubate for 5 min
- measure fluorescence intensity

LDH Assay

- add lysis buffer to control cells
- incubate for 1 h
- add substrate mix to all cells
- incubate for 30 min
- add stop solution
- measure absorbance intensity

Typical Results



Effects on cell metabolic activities, as determined by the conversion of MTS to formazan **(A)** and by measuring ATP concentration **(B)**, are used as indicators of cytotoxicity. TC_{50} represents the concentration causing a 50% cytotoxic effect.

References

1. Barile FA, Dierickx PJ and Kristen U (1994) *Cell Biol Toxicol* **10**: 155-162.

2. Davila JC, Reddy CG, Davis PJ and Acosta D (1990) *In vitro Cell Dev Biol* **26**:515-524.

3. Todd MD, Lin X, Stankowski LF, Desai M and Wolfgang GHI (1999) *J Biomolec. Screen* **4**:259-268.

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