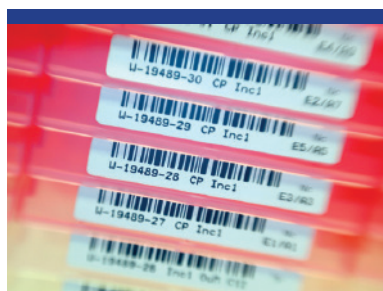# cyprotex
AN EVOTEC COMPANY

In vitro **Toxicology**

High Content Toxicology: Cytotoxicity Screening Panel

Background Information

'Assays with multiple parameters for key, multiple, and different features, such as in high content screening (HCS), are more predictive because they cover a wider spectrum of effects.'

[1]O'Brien P and Haskins JR (2007) *High Content Screening: A Powerful Approach to Systems Cell Biology and Drug Discovery* Ed. Taylor *et al*.; 415-425

- Drug toxicity is often a combination of multiple mechanisms. A single experimental approach is unlikely to capture the complexity involved in cellular toxicity.

- High Content Screening uses automated fluoresence imaging to simultaneously analyse multi-parametric indicators of cellular toxicity. It can detect general cell death and/or mechanisms of cell death within the same cell population within the same well, and it can cover a wide spectrum of cytopathological changes.

- Cyprotex have the most advanced High Content Screening equipment available, including four Thermo Scientific Cellomics ArrayScan® VTI's and a Thermo Scientific Cellomics ToxInsight

- Cyprotex offer a cytotoxicity panel to evaluate key toxicity markers including cell number, nuclear condensation, total nuclear intensity, cell permeability, mitochondrial membrane potential and cytochrome c release.

Protocol

Instruments
Cellomics ArrayScan® VTI or
Cellomics ToxInsight (Thermo Scientific)

Analysis Method
High Content Screening

Toxicity Markers
Cell loss
Nuclear size
Nuclear morphology
Cell membrane permeability
Mitochondrial membrane potential
Mitochondrial mass
Cytochrome c release

Cell Type
HepG2 (others available on request)

Test Article Concentration
8 point dose response curve up to 500 µM or solubility limit (different concentrations available)

Number of Replicates
3 replicates per concentration

Quality Controls
0.5% DMSO (vehicle control)
Chlorpromazine (positive control)
Valinomycin (positive control)

Test Article Requirements
3-5 mg solid (depending on molecular weight) or equivalent DMSO solution

Data Delivery
Minimum toxic concentration
Dose response curves

To find out more contact **enquiries@cyprotex.com**

The potential of HCS today lies in its versatility. HCS can be used for primary screening, basic research, target identification, biomarkers, cytotoxicity, and helping to predict clinical outcomes (Hoffman and Garippa, 2007).

Cells were incubated with a number of known toxic and non-toxic compounds at a range of different concentrations. At the end of the incubation period the cells were loaded with the relevant dye/antibody and scanned using an automated cell imager (Cellomics ArrayScan® VTI HCS Reader) to determine a panel of cell health markers. As expected all toxic compounds exhibited an effect on one or more endpoints whereas dexamethasone, a non-toxic compound, had no effect up to a concentration of 100 µM.

Figure 1

Simultaneous monitoring of multiple indicators of cell health in HepG2 cells using High Content Screening (HCS) technology. The images for treated cells (exposed to paclitaxel, cerivastatin or amiodarone over 24 hrs) are representative of an adverse effect on cell health as determined by monitoring nuclear morphology, cell membrane permeability, mitochondrial membrane potential and cytochrome c release. Data are compared with those from vehicle control wells (exposed to 0.2% DMSO over 24 hrs).
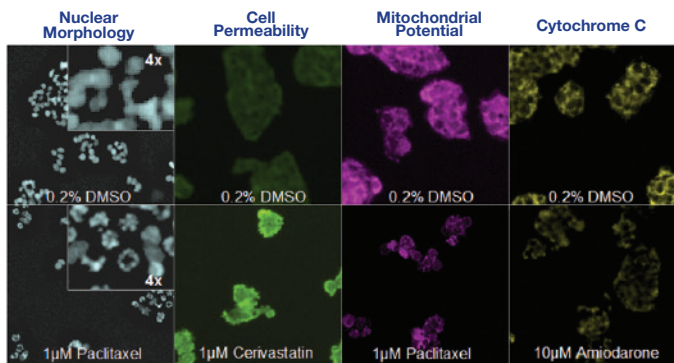
Using HCS technology, multiple observations can be detected from a single analysis. In the example in Figure 1, cell loss, nuclear size and nuclear intensity, cell membrane permeability, mitochondrial membrane potential and cytochrome c release from mitochondria were all observed in a single well.

Figure 2

Effect of different concentrations of amiodarone, paclitaxel and valinomycin on a panel of cytotoxicity markers.

Cell health parameter	Amiodarone				Paclitaxel				Valinomycin			
	vehicle	0.2µM	2µM	20µM	vehicle	0.01µM	0.04µM	0.4µM	vehicle	0.001µM	0.01µM	0.1µM
Nuclear intensity increase	Green	Red	Red	Red	Green	Green	Red	Red	Green	Green	Green	Green
Nuclear size decrease	Green	Green	Green	Red	Green	Green	Green	Green	Green	Green	Green	Green
Cell membrane permeability	Green	Green	Red	Red	Green	Green	Green	Red	Green	Green	Red	Red
Mitochondrial membrane potential	Green	Green	Green	Red	Green	Red	Red	Red	Green	Green	Red	Red
Cytochrome release	Green	Red	Red	Red	Green	Green	Green	Green	Green	Green	Green	Green
Cell loss	Green	Green	Green	Red	Green	Green	Green	Green	Green	Red	Red	Red

Green = No effect   **Red** = Toxic effect

In Figure 2, six different markers of cytotoxicity are evaluated, including nuclear size and intensity, cell membrane permeability, mitochondrial membrane potential, cytochrome c release and nuclear count. Using a concentration-dependent approach it is possible to distinguish early-stage events from later-stage events, and to uncover the mechanism of toxicity leading up to cell death. For example, in the case of amiodarone, cytochrome c release and nuclear intensity are observed at low concentrations (0.2 µM) and a greater range of cytotoxic effects, including cell membrane permeability and cell loss observed at higher concentrations (20 µM).

References

1. O'Brien P and Haskins JR (2007) High Content Screening: A Powerful Approach to Systems Cells Biology and Drug Discovery Ed. Taylor *et al*; 415-425
2. Hoffman AF and Garippa RJ (2007) High Content Screening: A Powerful Approach to Systems Cell Biology and Drug Discovery Ed. Taylor *et al*; 19-31