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In vitro Toxicology

Apoptosis and Necrosis Assay (Flow Cytometry)

Background Information

'Increased research on the mechanisms of cell death in recent years has led to the understanding that apoptosis and necrosis involve different cellular pathways and that these differences can have important implications when considering overall mechanisms of toxicity.'

[1] Elmore SA *et al.*, (2016) *Toxicologic Pathology* **44(2)**; 173-188

- Flow cytometry allows analysis of multiple parameters of cell health and provides a high throughput analysis of compound cytotoxicity.
- Apoptosis and necrosis occur during cell death in response to cytotoxic conditions. Cyprotex's apoptosis and necrosis assay utilises Annexin V and propidium iodide dual staining to monitor cellular death.
- Annexin V-FITC binds to phosphatidylserine which is translocated from the inner to outer plasma membrane during early apoptosis. Propidium Iodide is a cell impermeable nuclear dye which is excluded by viable and early apoptotic cells. However, it is taken up by necrotic or late apoptotic cells resulting in red fluorescence.
- A dose dependent increase in apoptotic or necrotic cells, accompanied by a decrease in live cells, indicates a compound is inducing cellular death.

Protocol

Cell Type
TK6, CHO-K1 (other lines available on request)

Analysis Platform and Method
Intellicyte iQue Screener PLUS

Test Article Concentrations
5-10 mg solid or equivalent solution

Time Points
Dosing time 24 hours (other time points available on request)

Quality Controls
Assay appropriate control

Endpoints
Quantification of live, dead and early apoptotic cells

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

Figure 1

Flow cytometric analysis of TK6 cells following treatment with etoposide.

TK6 cells were treated with etoposide for 24 hours prior to flow cytometric analysis using the apoptosis and necrosis assay. Live, dead and apoptotic populations were identified by plotting Annexin V intensity against PI intensity.

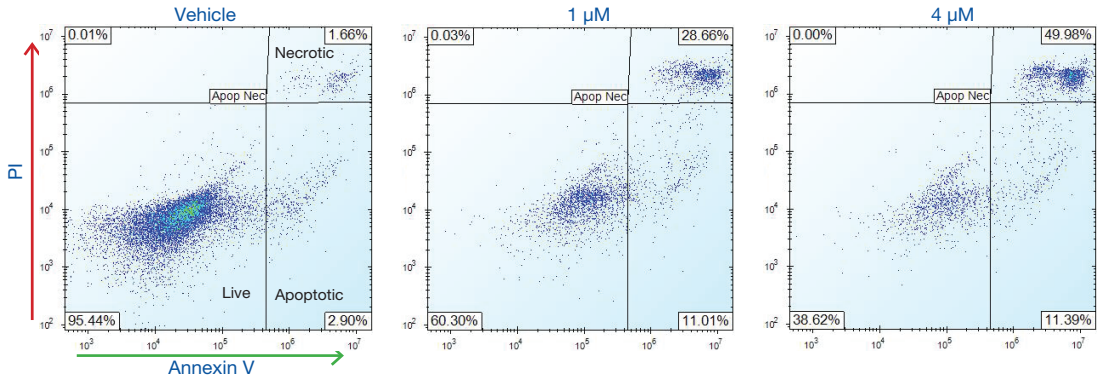
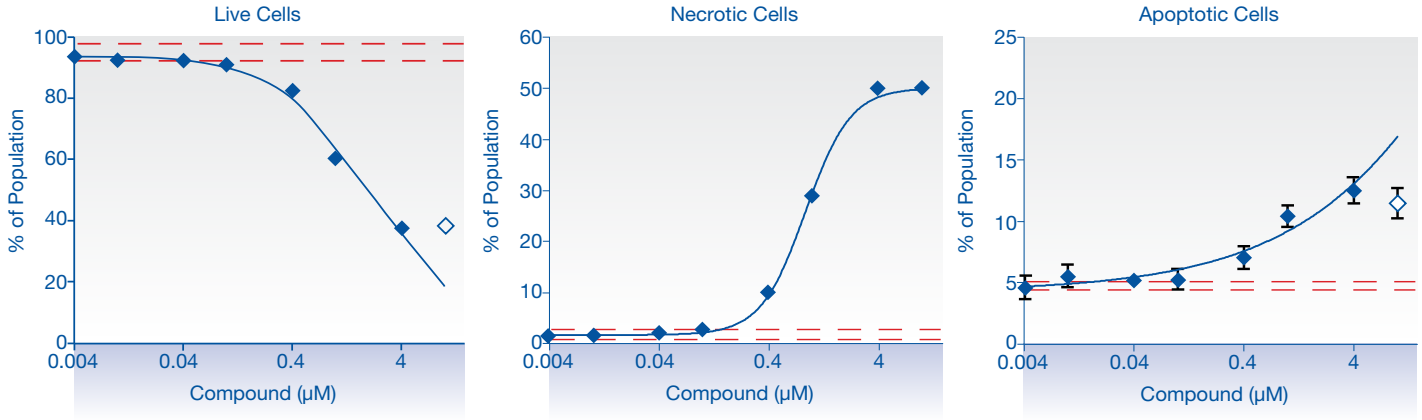


Figure 2

Graphical representation of flow cytometry data



TK6 cells were treated with etoposide for 24 hours followed by analysis using the apoptosis and necrosis assay. The percentage of apoptotic and dead cells increased in a dose dependent manner while the live cell percentage decreased proportionally.

Table 1

Summary of validation data

Compound	Cytotoxicity	Live		Apoptotic		Necrotic	
		MEC	MR (%)	MEC	MR (%)	MEC	MR (%)
Rotenone	cytotoxic	2.18	41.1	1.21	15.8	<0.04	51.5
Cyclosporin A	cytotoxic	1.88	32.5	0.707	54.2	0.868	49.2
Etoposide	cytotoxic	0.538	37.4	0.131	12.5	0.0417	50.1
Sunitinib	cytotoxic	13.7	9.7	45.8	30	7.38	84.6
Imatinib	cytotoxic	62.7	17.5	0.436	41.5	24.4	75.9
Carbonyl cyanide 3-chlorophenylhydrazone	cytotoxic	4.33	14.6	2.36	52.7	2.03	73.6
Chlorpromazine	cytotoxic	7.07	25.4	3.72	53.2	9.07	54.1
Chlorambucil	cytotoxic	9.61	14.1	NR	-	0.402	66.9
Amoxicillin	non-cytotoxic	NR	-	NR	-	NR	-
Alendronate	non-cytotoxic	NR	-	NR	-	NR	-

MEC- minimal effective concentration; MR- maximum observed response (% of total cells)

References

[1] Elmore SA *et al.*, (2016) Recommendations from the INHAND Apoptosis/Necrosis Working Group. *Toxicologic Pathology* **44(2)**; 173-188.