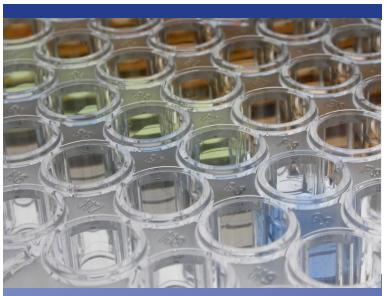
cyprotex AN EVOTEC COMPANY

In vitro **Toxicology**

In vitro Micronucleus Test (MNT) Flow cytometry TK6 cells

Background Information



'The *in vitro* MNT allows the detection of both clastogens and aneugens and it can simultaneously detect mitotic delay, apoptosis, chromosome breakage, chromosome loss and non-disjunction.'[1]

[1]Corvi R, Albertini S, Hartung T, Hoffmann S, Maurici D, Pfuhler S, van Benthem J and Vanparys P (2008) *Mutagenesis* **23(4)**; 271-283

- Two key classes of genotoxic agents are clastogens and aneugens. Clastogens directly damage DNA resulting in double strand DNA breaks. Aneugens produce numerical chromosome aberrations (aneuploidy), the result of "lagging" chromosomes.
- The actions of both anuegens and clastogens result in the formation of micronuclei within daughter cells following cell division.
- Flow cytometry allows multiparametric analysis of compound effects including cytostasis, cellular death and cell cycle arrest in addition to micronucleus formation.
- Flow cytometry quantifies marker intensity (Hoechst, CFDA-SE, EMA) and object size to detect micronuclei.
- Exclusion of necrotic and late apoptotic cells from analysis reduces false positives for genotoxicity. Monitoring of cytostasis and exclusion of cytostatic cells from micronucleus analysis also helps to reduce false negative results.
- The human lymphoblast line TK6 expresses the wild type p53 protein, shown to be important in accurate genotoxicity prediction[1].

Protocol

Test System
Flow cytometry using Intellicyte (iQue Screener Plus)

Cell Line
TK6 cells (other cells may be available on request)

Metabolising System
With or without S9 fraction

Time Points
3 hr (+S9) and 24 hr (-S9)

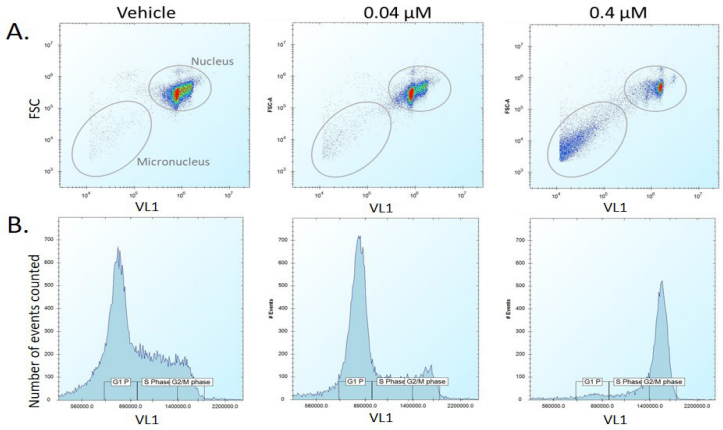
Quality Controls
Assay appropriate controls

Test Article Requirements
5-10 mg solid or equivalent solution depending on top test concentration required (200x to maintain 0.5% vehicle)

Endpoints
Micronucleus formation
Cell death
Cytostasis
Cell cycle analysis (G1, S, G2/M phase)

Figure 1

Representative data for micronucleus formation and cell cycle analysis.



Cells were treated with vehicle or paclitaxel at the stated concentrations for 24 hours prior to analysis. Micronuclei are detected as shown in figure 1A, with Hoechst staining (VL1) being utilised to identify the nuclei and micronuclei. The nuclei are then categorised as G1, S or G2/M phase (figure 1B).

Table 1

Summary of data for genotoxic and non-genotoxic reference compounds in the *in vitro* micronucleus test using TK6 cells and flow cytometry.

Compound	Category	-S9		+S9		Classification	
		MEC (µM)	Max response (% Control)	MEC (µM)	Max response (% Control)	-S9	+S9
Methyl methanesulfonate	Genotoxic	14.1	159	9.58	61.8	Positive	Positive
Etoposide	Genotoxic	0.51	102	1.13	73.8	Positive	Positive
Methotrexate	Genotoxic	<0.08	46.1	0.344	15.3	Positive	Negative
4-Nitroquinoline N-oxide	Genotoxic	0.0094	232	244	22.2	Positive	Negative
Mitomycin C	Genotoxic	<0.04	141	<0.04	266	Positive	Positive
Chlorambucil	Genotoxic	<0.2	123	<0.2	118	Positive	Positive
Cyclophosphamide	Genotoxic	NR	NR	2.93	261	Negative*	Positive
araC	Genotoxic	0.00329	55.9	0.0138	23.3	Positive	Negative
7,12-Dimethylbenz[a]anthracene	Genotoxic	0.24	55.3	0.869	87.3	Positive	Positive
Benzo[a]pyrene	Genotoxic	<1.56	61.3	<1.57	35.7	Positive	Positive
Nocodazole	Genotoxic	<0.04	228	41.4	192	Positive	Positive
Vinblastine	Genotoxic	<0.004	245	<0.004	18.5	Positive	Negative
Colchicine	Genotoxic	<0.004	203	0.0292	227	Positive	Positive
Paclitaxel	Genotoxic	<0.004	65.3	<0.004	291	Positive	Positive
Diethylstilbestrol	Genotoxic	0.962	195	31.7	2.88	Positive	Negative
Griseofulvin	Genotoxic	2.44	66.5	NR	NR	Positive	Negative
Dexamethasone	Non-genotoxic	27.2	1.45	NR	NR	Negative	Negative
Chlorpromazine	Non-genotoxic	NR	NR	NR	NR	Negative	Negative
CCCP	Non-genotoxic	1	3.68	34.3	3.52	Negative	Negative
Cyclosporin A	Non-genotoxic	NR	NR	NR	NR	Negative	Negative
Imatinib	Non-genotoxic	NR	NR	47.2	8.21	Negative	Negative
Staurosporine	Non-genotoxic	0.0165	9.23	1.22	33.2	Negative	Negative
Alendronate	Non-genotoxic	35.8	3.07	NR	NR	Negative	Negative
Amoxicillin	Non-genotoxic	NR	NR	NR	NR	Negative	Negative
Metformin	Non-genotoxic	NR	NR	NR	NR	Negative	Negative
Nalidixic acid	Non-genotoxic	NR	NR	NR	NR	Negative	Negative
Pyrene	Non-genotoxic	NR	NR	19	4.53	Negative	Negative

* Cyclophosphamide is metabolically activated.
NR = No Response.

TK6 cells were incubated with a panel of compounds in the presence or absence of S9 fraction for 3 or 24 hours respectively prior to analysis. At concentrations where over 30% cellular death or cytostasis was observed any present micronuclei was excluded. Compounds were categorised as genotoxic if they induce a 35 fold increase in micronucleus formation compared to vehicle only controls. Green boxes show correctly predicted compounds. The positive maximum (Max) response threshold was set at 35 fold increase compared to vehicle only controls. Orange boxes are above this threshold, blue boxes are below the threshold.

References

[1] Kumari R *et al*., (2014) *Mol Cell Oncol* **1(13)**; DOI: 10.4161/23723548.2014.969653
[2] Corvi R *et al*., (2008) *Mutagenesis* **23(4)**; 271-283