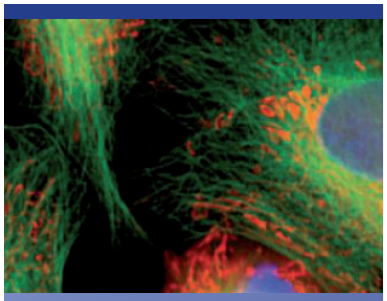
cyprotex AN EVOTEC COMPANY

In vitro **Toxicology**

In vitro HCS Micronucleus Test (MNT)

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

Related Services

- GreenScreen HC™
- Ames Test

- Cyprotex offer the *in vitro* micronucleus test using fluorescent cellular imaging, high content screening (HCS) and an ArrayScan® VTI HCS reader (Thermo Scientific Cellomics).
- High content screening (HCS) has several advantages over traditional manual methods – these include increased throughput, the removal of subjectivity through automated scoring, and the rapid measurement of large numbers of cells which increases statistical power.
- During cell division, if a chromosome or a fragment of a chromosome is not incorporated into one of the daughter nuclei, typically as a consequence of genetic damage, it may form its own "micronucleus".
- Using HCS the *in vitro* micronucleus test simultaneously collects multiple endpoints including, relative survival (cell count), membrane integrity (cytotoxicity assessment) and cell cycle information (binucleated cell frequency and proliferation index (CBPI)). In combination these parameters determine cell health (cytostasis) and micronuclei validity.
- The assay delivers rapid multi-parametric assessment with the benefit of low compound requirements. In addition, the assay can detect genotoxins requiring metabolic transformation by utilising Aroclor-treated rat liver S9.
- The micronucleus test complements other genotoxicity assays to provide a more mechanistic insight into genotoxicity. It also acts as a bridge to the *in vivo* micronucleus test required in the ICH guidelines.

Protocol

Test System
Micronucleus test using fluorescent cellular imaging, high content screening (HCS) and an ArrayScan® VTI HCS reader (Thermo Scientific Cellomics)

Cell Line
CHO-K1 cells

Test Article Concentrations
10 serial dilutions, test compound concentrations (depending upon solubility) 500, 200, 50, 20, 5, 2, 0.5, 0.2, 0.05, 0.02 µM, final DMSO concentration = 0.5 %

Test Article Incubation Time
In absence of S9: 24 hr exposure time
In presence of S9: 3 hr exposure, followed by 21 hr recovery time

Cytokinesis Block Method
Following compound exposure cytokenesis is blocked (27.5 hr).

Quality Controls
0.5 % DMSO - negative vehicle control
In absence of S9: Griseofulvin (Aneugen) - positive control
In presence of S9: Cyclophosphamide (Clastogen) - positive control.[2]
2000 binucleated cells measured per concentration.[2]

Test Article Requirements
100 µL of a 100 mM DMSO solution or equivalent amount in solid compound.

Metabolising System
Aroclor-1254 induced rat liver S9

Data Delivery
Written report presenting overall results
Minimum effective concentration (MEC), maximum response, fold change and % change for observed micronuclei above vehicle control
Graphical representation of data for genotoxicity (targeted cells with micronuclei %) and cytotoxicity (relative survival, binucleated cell frequency, CBPI, and cytostasis).

‘In recent years the *in vitro* micronucleus test has become an attractive tool for genotoxicity testing because of its simplicity of scoring and wide applicability in different cell types.’[3]

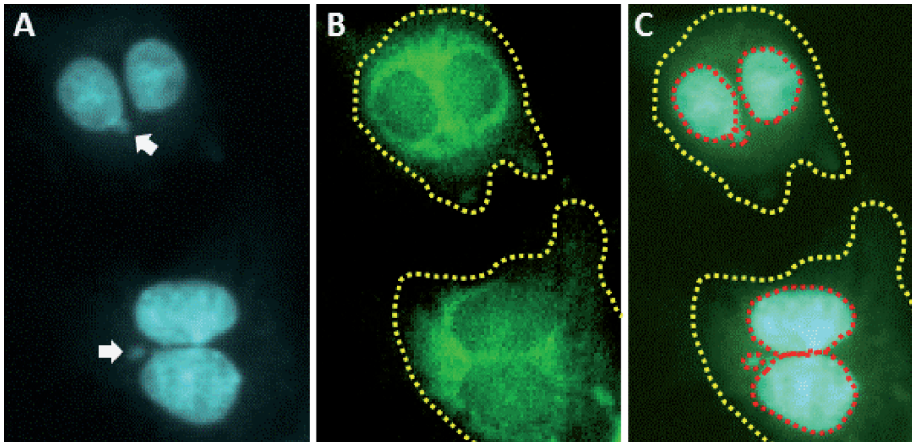


Figure 1

Determining micronuclei using high content screening: A) Nuclear dye stains binucleated cells and micronuclei (white arrows), B) Cellular dye ensures only single cells are selected, C) The composite image and membrane permeability dye ensures cell health and correct micronuclei identification.

The Cyprotex *in vitro* micronucleus assay (MNT) has been validated using a number of different genotoxic and non genotoxic compounds.

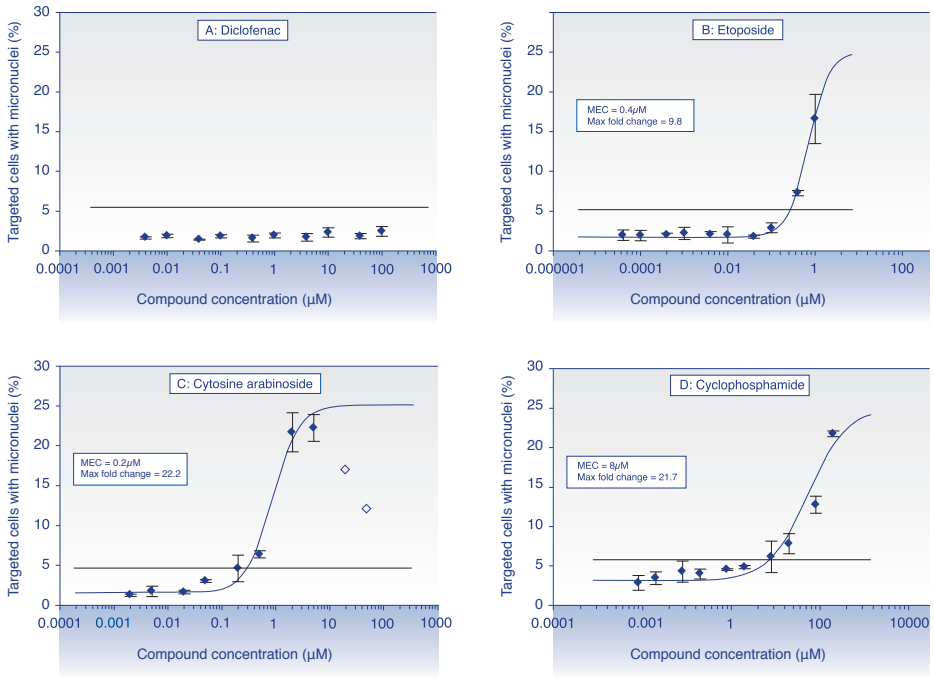


Figure 2

Graph illustrating % micronuclei within targeted cells (min. 2000) data from the Cyprotex *in vitro* micronucleus test for non-genotoxic (A: diclofenac), aneugen (B: etopsode; -S9), clastogen (C: cytosine arabinoside; -S9) and indirect clastogen (D: cyclophosphamide; +S9) compounds (mean ± sd; n=2). Filled diamonds represent valid micronuclei (above 3 fold vehicle control (black line) is a positive response). Open diamonds are invalid micronuclei due to cell heath criteria.

The MEC values displayed on the graphs represent the minimum effective concentration of drug at which a positive micronucleus result is observed (greater than 3 fold over vehicle control). Criteria are used to identify a positive result in the micronucleus test based on relative survival (cell count), membrane integrity (cytotoxicity assessment) and cell cycle information (binucleated cell frequency and proliferation index (CBPI)). In combination these parameters determine cell health (cytostasis) and micronuclei validity.

References

[1] Corvi R *et al*, (2008) ECVAM retrospective validation of *in vitro* micronucleus test (MNT). *Mutagenesis* **23(4)**; 271-283

[2] OECD Guidelines for the testing of chemicals: *In vitro* Mammalian cell micronucleus test, July 2010 (#487)

[3] Decordier I and Kirsch-Volders M (2006) The *in vitro* micronucleus test: From past to future. *Mutation Research* **607**; 2-4