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In vitro **Toxicology**

In vitro Comet Assay

Background Information

'In addition to the capability of the Comet assay to identify DNA damage at the single cell level, other significant advantages include its sensitivity for detecting low levels of DNA damage, the requirement for only small numbers of cells per sample, its ease of application and the short time needed to perform the assay.'

[2]Liao W, McNutt MA and Zhu W-G (2009) *Methods* **48**; 46-53

- Cyprotex have partnered with Gentronix, specialists in genotoxicity screening, to offer the *in vitro* Comet assay.
- The Comet assay, also called the single cell gel electrophoresis (SCGE) assay is a sensitive, rapid and relatively simple method for detecting DNA damage at the individual eukaryotic cell level[1].
- Electrophoresis is performed on exposed cells that have been embedded in agarose microgels. Damage resulting in DNA fragmentation is detectable by migration away from the nucleus giving a comet-like appearance with the head consisting of intact DNA and the tail consisting of damaged DNA.
- The assay delivers rapid and sensitive DNA damage assessment with the benefit of low compound requirements.
- The Comet assay complements other genotoxicity assays to provide a mechanistic insight into genotoxicity.

Protocol

Test System
Alkaline Comet (single cell gel electrophoresis)

Cell Line
TK6 cells

Test Article Concentrations
5 serial dilutions, typically 2-fold; e.g. 1000, 500, 250, 125, 62.5 µg/mL

Incubation Time
3 hr exposure time

Quality Controls
1 % DMSO (negative control)

Etoposide (positive genotoxic control)

Cell Viability Assessment
Propidium iodide exclusion using flow cytometry; 80% relative survival required for Comet processing

Test Article Requirements
10 mg of solid compound — Other compound preparations are acceptable

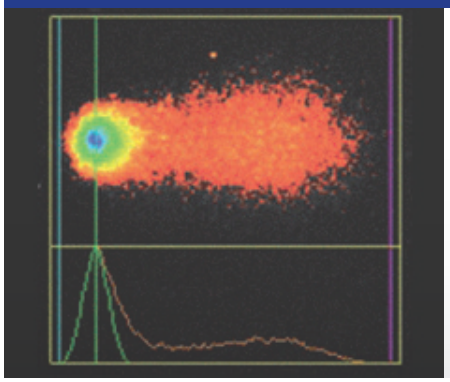
Data Analysis
50 comets per compound dose acquired from two agarose gels (25 from each gel)

Data Delivery
Written report presenting overall results

Excel worksheets with full graphical dose response data for genotoxicity including % tail intensity

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

The comet assay is a versatile and sensitive method for measuring single- and double-strand breaks in DNA.[3]

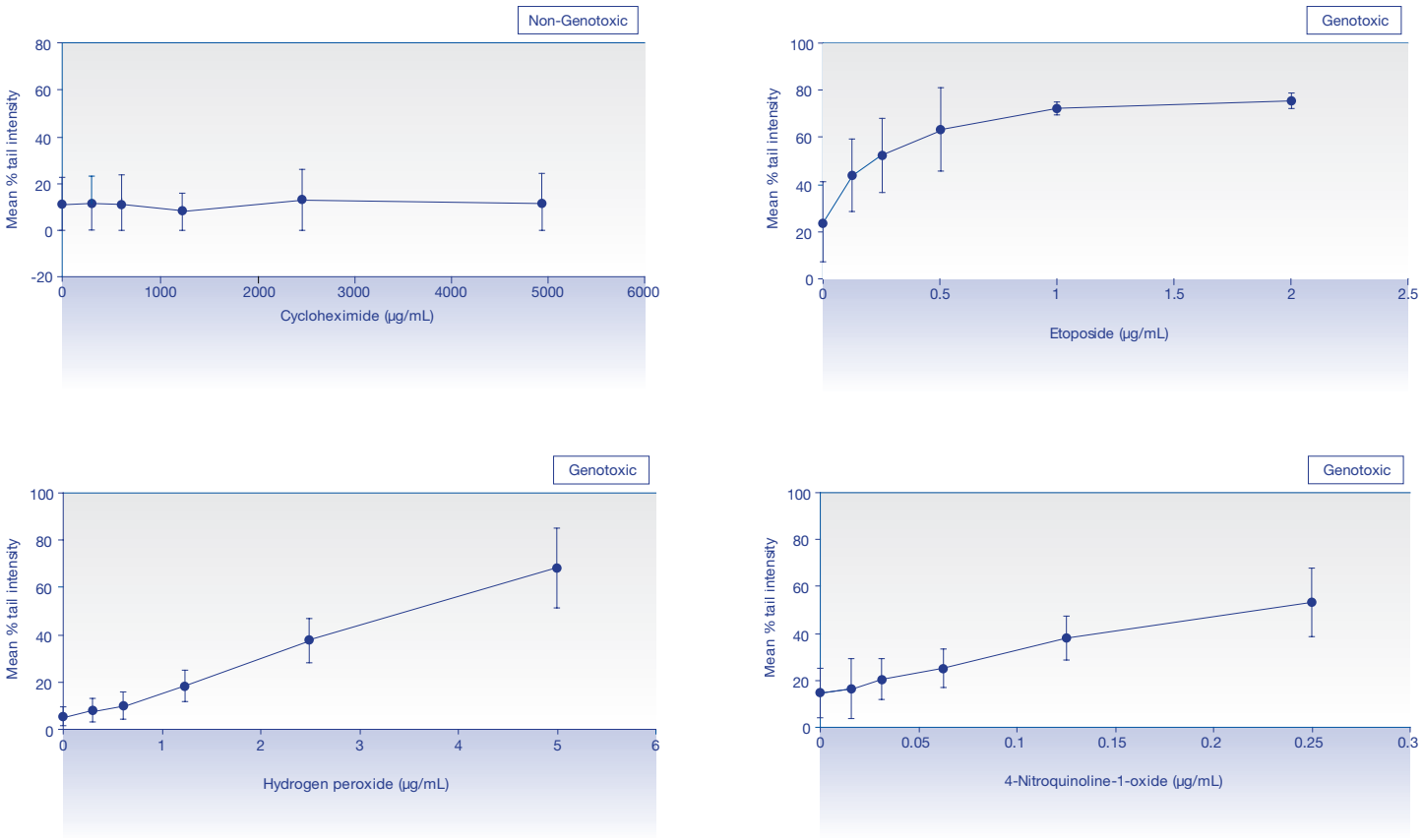


In vitro Comet Assay

The *in vitro* Comet assay has been validated against a number of different genotoxic and non-genotoxic compounds.

Figure 1

Graphs illustrating mean tail intensity data from the Comet assay for non-genotoxic (cycloheximide) and genotoxic (etoposide, hydrogen peroxide and 4-nitroquinoline-1-oxide) compounds (mean ± sd; n=3).



References

[1] Singh NP *et al*, (1988) *Exp Cell Res* **175**; 184-191
[2] Liao W *et al*, (2009) *Methods* **48**; 46-53
[3] Collins AR *et al*, (2008) *Mutagensis* **23(3)**; 143-151