The Comet Assay

The comet assay is increasingly used in industrial genotoxicity in vitro. During early drug development, robust genotoxicity screening assays are required that reliably predict the outcome of time- and resource consuming regulatory tests. In this respect the comet assay is a promising tool because the test is rapid, easy to perform and requires only minute amounts of test substance.

1. Introduction

The single cell gel electrophoresis (SCGE) assay commonly referred to as "comet assay" allows the very sensitive detection of DNA breakage induced by genotoxic agents at single cell level. Due to its sensitivity and simplicity, the comet assay provides a broad platform for mutagenic testing as well as for apoptosis investigations.

2. Principle of test method

Cell cultures (V79^{TM}) are exposed to the test substances both with and without metabolic activation for 3 or 6 hours. After exposure to a test substance, cell are embedded in agarose on a microscope slide, lysed with detergent and after a denaturation step during electrophoresis, DNA fragments as outcome of single-stranded and double-stranded breaks migrate faster in the electric field than intact DNA. If the DNA is stained with an appropriate fluorochrome (ethidiumbromide) the eluted fragments are visible as comet tail under fluorescence microscopy conditions. The relative intensity of fluorescence in the tail is a function of the frequency or DNA breaks; it can be assessed either visually or using densitometry and computer-based analysis.

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3. Exposure concentrations

In consideration of solubility and cytotoxicity the highest test item concentrations are 10 mM, 5 mg/ml and 5 µl/ml. At least five analysable concentrations are tested.

4. Controls

Cells are tested with the solvent or vehicle as the negative control and with known strand break inducing substances as positive control both with and without metabolic activation.

positive controls	w/o metabolic activation	w metabolic acitvation
	N-methyl-N'-nitro-N-nitrosoguanidine methyl methanesulphonate	7,12 dimethylbenzanthracene cyclophosphamide 2-aminoanthracene benzo[a]pyrene

5. Evaluation/Analysis

Visual scoring entails categorizing randomly selected comets according to relative tail intensity; 100 comets are sorted into five classes and assign a value to its class, so that a single overall rating for the slide between 0 and 400 (100000) can be obtained by summation. The visual classes correspond very roughly to 20% intervals for the computer assessed scores of percent DNA in tail.

6. Interpretation of results

A criteria for a positive result is a concentration-related increase in DNA migration and a significant corresponding increase in DNA migration at one or more dose groups.

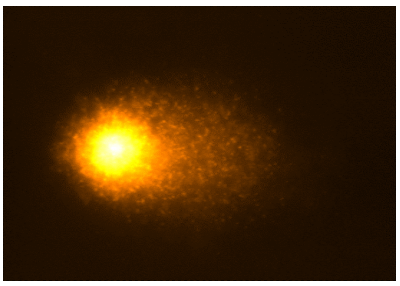
Positive results from the in vitro comet assay indicate that the test substance induces DNA damage in cultured cells.

If there is a clearly positive result there is no requirement for further testing. Equivocal results are clarified by further testing. Negative results need to be confirmed.

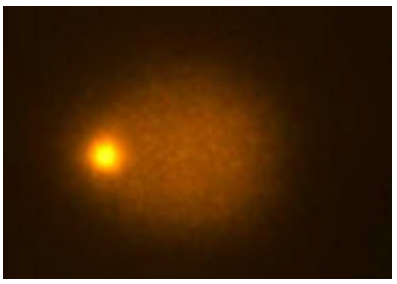
7. References:

- Tice R, Agurell D, Anderson D, Burlinson B, Hartmann A., Kobayashi H, Miyamae, Rojas E, Ryu J, Sasaki Y: The single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing; Environmental Molecular Mutagenesis 35: 206-221 (2000)
- Olive P, Banath J, Durand R: Detection of Etoposide resistance by measuring DNA damage in individual Chinese hamster cells. Journal of the National Cancer Institute 82: 779-783 (1990)

cell without DNA fragmentation



cell with DNA fragmentation



cell with major DNA-fragmentation (positive control)

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