

# Alkaline In Vitro Comet Assay in C3a cells

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## SCOPE OF THE METHOD

<b>Alternative method relates to</b>	Human health
<b>Alternative method is situated in</b>	Basic Research
<b>Type of alternative method</b>	In vitro - Ex vivo
<b>This method makes use of</b>	Human derived cells / tissues / organs
<b>Specify the type of cells/tissues/organs</b>	C3a Cells (Hepatocellular Carcinoma Cells, the C3A cell line is a clonal derivative of Hep G2 cells )

## DESCRIPTION

### Method keywords

DNA damage

single cell gel electrophoresis assay

comet

### Scientific area keywords

genotoxicity

### Method description

The Alkaline Comet Assay is a microgel electrophoresis technique which allows to measure DNA damage (single and double strand breaks, alkali labile sites, incomplete excision repair sites and cross links) cell by cell. Cells are mixed with 0.8% Low Melting

Point Agarose which is spread as a gel onto a microscope slide. The cells are lysed with high salt concentrations and detergents. The remaining nuclear DNA is then denatured in alkali buffer  $\text{pH} > 13$  and electrophoresed in the same buffer. The DNA fragments migrate out of the nucleus, towards the positive pole whereas undamaged supercoiled DNA won't migrate. After electrophoresis and neutralization of the slides, cells are dried and stained with GelRed, a fluorochrome intercalating agent. A fluorescent microscope equipped with an image analysis system is used to capture and quantify DNA damage in the single cells. DNA damage is expressed as percentage of DNA in the tail.

### **Lab equipment**

Electrophoresis Chamber with Power Supply Circulating Pump

Fluorescence Microscope

Software for automated imaging

### **Method status**

History of use

### **PROS, CONS & FUTURE POTENTIAL**

#### **Advantages**

Identify DNA damage at the single cell level

Sensitivity for detecting low levels of DNA damage

Requirement for only small numbers of cells per sample

Fast, cheap & easy

Applicable on many cell types

#### **Challenges**

Most of the damage is repaired (no fixed mutations are detected, regulatory genotoxicity testing in vitro usually relies on mutagenicity test not indicator tests ) but this gives an opportunity to study DNA repair

Quality of protocol and experimental performance is of crucial importance

Suitable statistical analysis

Incomplete metabolic capacities C3a cell line (Problems with indirect mutagens)

### **Modifications**

Use of a cooling system for the electrophoresis device to minimize slide/slide differences

Improvement of the imaging system (upgrade software metasystems)

### **Future & Other applications**

High Throughput analysis (48 well slides Trevigen)

Use of lesion specific enzymes to make comet more sensitive (eg FPG) and more specific (eg epigenetic studies)

## **REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION**

### **References**

Collins AR, Azqueta Oscoz A, Brunborg G, Gaivão I, Giovannelli L, Kruszewski M, Smith CC, Stetina R (2008) The comet assay: topical issues. *Mutagenesis* 23:143–151

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen* 35:206–221

Azqueta A. and Collins A. R . (2013) The essential comet assay: a comprehensive guide to measuring DNA damage and repair. *Arch. Toxicol .*, 87, 949–968

Investigation into the genotoxicity of water extracts from Hypoxis species and a commercially available Hypoxis preparation., Verschaeve, Luc, Mertens Birgit, Ndhlala A R., Anthonissen R, Gorissen B, and Van Staden J , *Phytother Res*, 2013 Mar, Volume 27, Issue 3, p.350-6, (2013)

### **Associated documents**

[The essential comet assay a comprehensive guide to measuring DNA damage and repair.pdf](#)

[Single Cell GelComet Assay Guidelines for In Vitro and In Vivo Genetic Toxicology Testing.PDF](#)  
[the comet assa topical issues Mutagenesis-2008-Collins-143-51.pdf](#)  
[Investigation into the genotoxicity of water extracts from Hypoxis species and a commercially available Hypoxis preparation.pdf](#)

### Other remarks

Apart from the standard Comet assay for genotoxicity testing, in our laboratory we examine also the antigenotoxic properties of test substances (mostly plant extracts). A test solution is considered antigenotoxic when the genetic damage caused by the combined treatments (extracts and known mutagen) is substantially lower compared to the damage induced by the mutagen alone.

## PARTNERS AND COLLABORATIONS

### Organisation

**Name of the organisation** Sciensano

**Department** Chemical and physical health risks

**Specific Research Group or Service** Risk and health impact assessment

**Country** Belgium

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