Alkaline In Vitro Comet Assay in C3a cells

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SCOPE OF THE METHOD

Alternative method relates to	Human health
Alternative method is situated in	Basic Research
Type of alternative method	In vitro - Ex vivo
This method makes use of	Human derived cells / tissues / organs
Specify the type of cells/tissues/organs	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 desription

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 denaturated in alkali buffer 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 a 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 epigenitic 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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