Measurement of the extracellular release of adenosine triphosphate in cultured primary rat hepatocytes

Commonly used acronym: *ATP measurement*
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SCOPE OF THE METHOD

The Method relates to	Animal health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
This method makes use of	Animal derived cells / tissues / organs
Species from which cells/tissues/organs are derived	rat
Type of cells/tissues/organs	primary hepatocytes

DESCRIPTION

Method keywords

ATP

cytotoxicity

bioluminescent determination

extracellular ATP

Scientific area keywords

Hepatotoxicity
liver
cholestasis
Steatosis

Method description

ATP transports chemical energy within cells by serving as a substrate for kinases and as such fulfills a vital function in numerous cellular processes such as cell injury and subsequent cell death. ATP is therefore a crucial player in these events that are results of intracellular stress. Hepatotoxic chemical compounds can cause intracellular stress. The general cytotoxicity of compounds can be estimated through the biolouminescent assessment of extracellular release of ATP. As such, this procedure relies on two reactions in which firefly luciferase catalyzes the oxidation of luciferin to oxyluciferin and whereby ATP is consumed and light becomes emitted. The latter can be measured and is proportional to the amount of ATP present outside cells.

Lab equipment

Multiplate reader (Victor, 1420 Multilabel counter, PerkinElmer, Belgium)

Method status

History of use

PROS, CONS & FUTURE POTENTIAL

Advantages

Easy to apply method to quantitatively characterize extracellular ATP release and hence cell injury in primary hepatocyte cultures.

Challenges

Increased extracellular levels of ATP do not specifically indicate cell death by either apoptosis or necrosis. Ideally this method should be combined with established tests, such as the monitoring of cell proliferation potential and mitochondrial function, which can be done by measurement of the incorporation of 5-bromo-2'-deoxyuridine

(BrdU) during DNA syntehsis and by adressing an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, respectively.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

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Associated documents

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