

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

**Commonly used acronym:** ATP measurement

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## Organisation

**Name of the organisation** Vrije Universiteit Brussel (VUB)

**Department** Pharmaceutical and Pharmacological Sciences

**Specific Research Group or Service** In Vitro Toxicology and Dermato-Cosmetology

**Country** Belgium

**Geographical Area** Brussels Region

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Animal health
<b>The Method is situated in</b>	Basic Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Species from which cells/tissues/organs are derived</b>	rat

<b>Type of cells/tissues/organs</b>	primary hepatocytes
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## 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 bioluminescent 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 synthesis and by addressing an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, respectively.

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

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