

## Measurement of reactive oxygen species in cultured primary rat hepatocytes

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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

### 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>	Hepatocytes

### DESCRIPTION

#### Method keywords

Hepatocytes  
Hepatotoxicity  
cells  
rat  
rodent  
ROS  
reactive oxygen species  
DCFH-DA

#### Scientific area keywords

hepatic toxicity  
hepatology  
hepatocytes  
toxicity testing  
toxicity

#### Method description

Basically, the standard operating procedure outlined in this document consists of the following steps, namely, (i) preparation of the cells for the DCFH-DA assay, (ii) DCFH-DA

test procedure, (iii) processing of the results. Practical details are provided for each of these steps and are followed by some useful tips based upon our own hands-on experience. The fluorescent assay DCFH-DA is based on the ability of the non-fluorescent lipophilic DCFH-DA probe to penetrate viable cells, where it is being deacetylated by the presence of intracellular esterases and trapped within the cells. Upon exposure of the cells to a stimulus that generates the oxygen metabolic burst and subsequently the production of ROS, the probe is oxidized and starts to emit an intense green fluorescence. This assay allows the determination of the oxidative stress induction potential of chemical substances. As such, after incubation with the probe, the cells are exposed to the selected substances and the respective emitted fluorescence can be measured for a period of 30 min. The increase in fluorescence is proportional to the amount of ROS that are being formed within the cells. The results can be read by the use of a multiwell scanning fluorimeter (plate reader) using an 485-495nm emission filter and an 520-530nm excitation filter. ROS production is expressed as a ratio of treatment versus control. This method provides a sensitive, reproducible and integrated signal of both attached cells and cells in suspension.

### **Lab equipment**

Multiwell scanning fluorimeter (plate reader) using an 485-495nm emission filter and an 520-530nm excitation filter ;  
Laminar flow cabinet ;  
Thermostated bath.

### **Method status**

History of use  
Internally validated

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

### **Advantages**

The method is sensitive, reproducible and capable of giving an integrated signal of both attached cells and cells in suspension.

### **Challenges**

Unequally seeded cells can give divergent testing results. Take care to handle the seeded cells with caution. Air bubbles in the wells of the 96-well plate can cause negative testing results, gently shake the plate before putting it into the plate reader to avoid this.

### **Future & Other applications**

Can be applied to other cell types ;  
Possible modification needed.

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

### **References**

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Burchardt P., Warowicka A., Gozdicka-Józefiak A. and Wysocki H. (2010) Disturbances of mitochondrial energetic processes and mt-DNA and their role in the etiology of coronary artery disease. *Kardiologia Polska* 68(8): 947-950

Hamanaka R.B. and Chandel N.S. (2010) Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. Trends in Biochemical Sciences 35(9): 505-513

Keston S. and Brandt R. (1965) The fluorometric analysis of ultramicro quantities of hydrogen peroxide. Analytical Biochemistry 11: 1–5

### **Associated documents**

[ROS assay.doc](#)

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