

## Sudan Red III in situ staining of 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

**Geographical Area** Brussels Region

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research, Translational - Applied 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 rat hepatocytes

## DESCRIPTION

### Method keywords

Formaldehyde fixation  
Sudan Red III staining  
Hematoxylin nuclear counterstain  
Primary rat hepatocytes  
Intracellular lipids  
in vitro

### Scientific area keywords

Toxicology  
Hepatotoxicity  
Steatosis  
Drug-induced cytotoxicity

### Method description

The standard operating procedure for Sudan Red III in situ staining of cultured rat hepatocytes describes how to detect one of the aspects of drug-induced cytotoxicity i.e. the intracellular accumulation of lipids or in other words steatosis, in primary rat hepatocyte cultures. It is based on the ability of a lysochrome, i.e. Sudan Red III diazo-dye to stain intracellular lipids. Additionally, subsequent application of hemalum, which is a complex formed by aluminium ions and oxidized haematoxylin, colours nuclei of the cells and thus enables their localisation. Red-coloured lipid droplets and blue nuclei are readily visible upon examination of the cells under a light microscope.

### Lab equipment

Inverse-phase light microscope (Nikon Optiphot);  
Oven (Thermo electron corporation, Heraeus, 60°C).

## PROS, CONS & FUTURE POTENTIAL

### Advantages

The standard operating procedure for Sudan Red III in situ staining of cultured primary rat hepatocytes is easily applicable and allows a simultaneous screening of multiple compounds and/or multiple concentrations of the same compounds (to examine chemically induced steatosis).

### Challenges

Sudan Red III stain has a high affinity to a broad range of lipids and consequently does not discriminate between e.g. neutral lipids and phospholipids. Therefore, it is of utmost importance to perform more than one assay or use a more specific assay.

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### Associated documents

[Sudan Red III staining.doc](#)

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