Sudan Red III in situ staining of cultured primary rat hepatocytes


Contact person
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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

<table>
<thead>
<tr>
<th>The Method relates to</th>
<th>Human health</th>
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</thead>
<tbody>
<tr>
<td>The Method is situated in</td>
<td>Basic Research, Translational - Applied Research</td>
</tr>
<tr>
<td>Type of method</td>
<td>In vitro - Ex vivo</td>
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<tr>
<td>Species from which cells/tissues/organs are derived</td>
<td>Rat</td>
</tr>
<tr>
<td>Type of cells/tissues/organs</td>
<td>Primary rat hepatocytes</td>
</tr>
</tbody>
</table>

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